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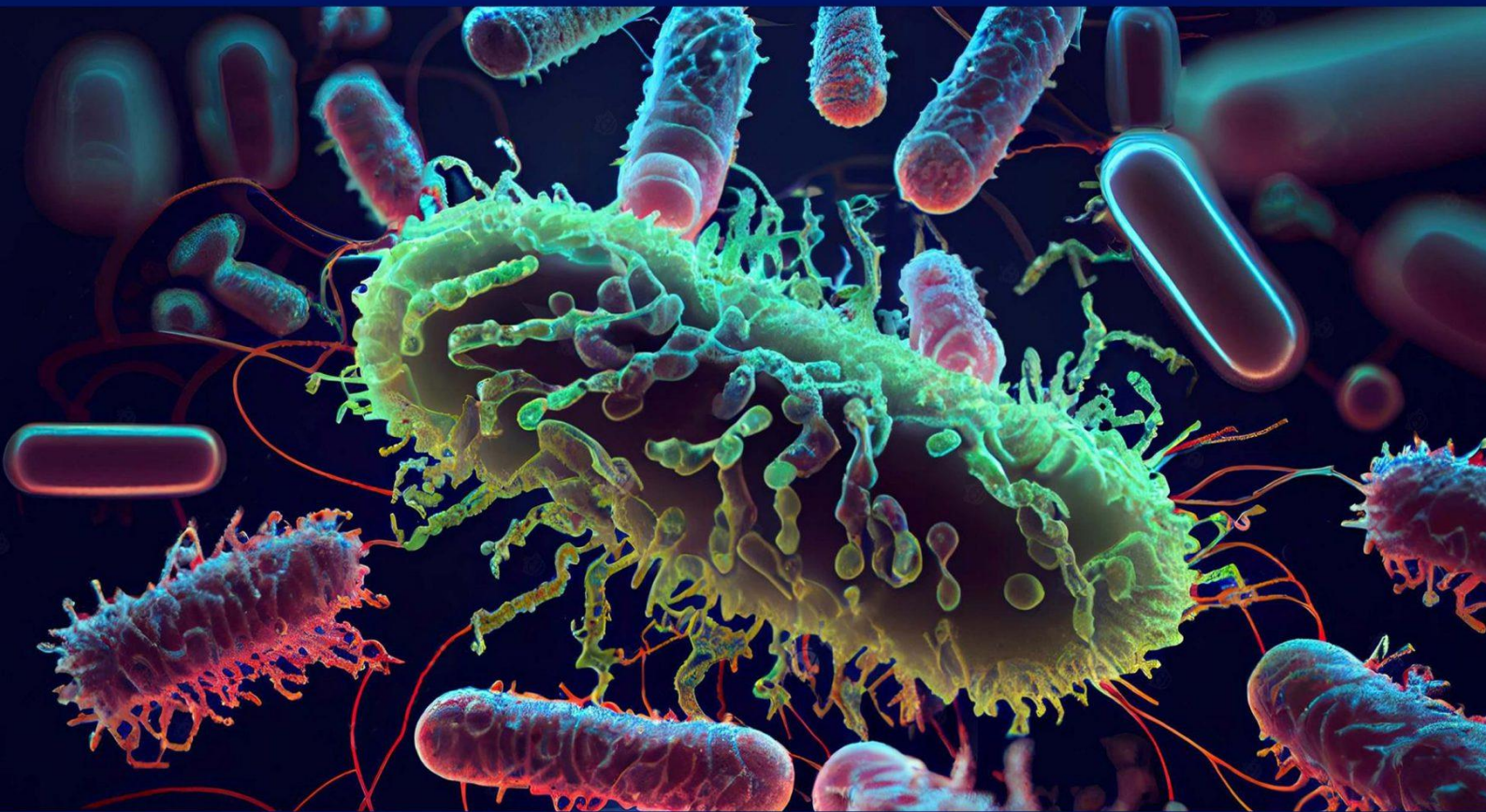
**Deadline: July 22, 2023**



# **24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY**

## **SEPTEMBER 18-20 / TEHRAN, IRAN**

**Iranian Research Organization for Science and Technology (IROST)**



## **Advanced Innovations in Microbiology and Research on Industrial Microbes**



**IROST**



**Iranian Research Organization for Science and Technology (IROST), Sh.Ehsani Rad St., Enqelab St., Ahmadabad Mostoufi Rd., Azadegan Highway.**

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Iranian Research Organization for  
Science and Technology



**24<sup>th</sup> IRAN'S INTERNATIONAL  
CONGRESS OF MICROBIOLOGY**



Iranian Society of  
Microbiology

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Iranian Research Organization for  
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**24<sup>th</sup> IRAN'S INTERNATIONAL  
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Iranian Society of  
Microbiology

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**President of Iranian Society of Microbiology**



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**General and Industrial Scientific Secretary**



Prof. Fereshteh Shahcheraghi

**Medical Microbiology Scientific Secretary**



Dr. Maliheh Safavi

**Executive Secretary**



## ISM's President Welcome



Dear All ISM members, the honorable guests and invited speakers

Welcome to our 24<sup>th</sup> International Congress of Microbiology. The 24<sup>th</sup> meeting is a special event since the Microbiologists with different research background from all provinces will get together at IROST. The conclusive topics cover all areas that is related to Microbiology. Because of diversity in subjects, we have two Professors in charge of scientific secretary: one in Medical Microbiology and the second one in field of applied and General Microbiology. Our colleagues, particularly the young scientists and post-graduate students always have been active and participate in our annual meetings. The 24<sup>th</sup> congress will provide more opportunities to them to exchanges their views and news on the progress in this important subject in our country as well as other part of the world. Moreover, it favors the producers and researchers to benefit from the facilities that IROST provide them to debate about the possibilities for collaborations. The end I would like to thanks all the participants who submitted their research results, the invited speakers, the panelist and exhibitors who creates a very scientific and nice gathering in ISM 2023 meeting.

I wish you a pleasant stay in Tehran and enjoy the program at the IROST campus



## Scientific Committee

### Medical Microbiology

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31	Khorshidi, Ahmad	Kashan University of Medical Sciences
32	Khosravani, Seyed Abdolmajid	Yasouj University of Medical Sciences
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36	Mansouri, Shahla	Kerman University of Medical Sciences



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32	Dr. Mohammadi, Parisa	Alzahra
33	Dr. Mollasalehi, Hamidreza	Beheshti University





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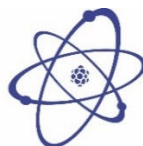
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## Keynote Speakers

### International Invited Speakers



**Tissue sampling with infrared-laser systems – getting closer to the original composition of molecules in tissues**

**Dr. Maria Riedner**

Coordinator of the Technology Platform Mass Spectrometry  
University of Hamburg  
Hartmut Schlüter's Lab  
Hamburg, Germany



**Analysis of proteoforms – closer to the relationship of proteins and their functions**

**Professor Hartmut Schlüter**

Professor of University Medical Center Hamburg-Eppendorf  
Member of the Board of Directors of the Consortium for Top-Down Proteomics (CTDP)  
Head of Section "Mass Spectrometric Proteomics" of the UKE



**Strategies of Antimicrobial Stewardship**

**Professor Cristina Mussini**

Professor of Infectious Diseases at the University of Modena and Reggio Emilia, Italy  
Director, Clinic of Infectious Diseases, Azienda Ospedaliero-Universitaria Policlinico, Modena, Italy



**The Importance of Fast Microbiology in the Antimicrobial stewardship era**

**Professor Stefania Stefani**

Professor of Microbiology and Clinical Microbiology.  
President of the Italian Society of Microbiology (SIM)



**Strategies of Infectious Control**

**Dr. Marianna Maschiarì**

Coordinator of Antimicrobial Stewardship Group and Active Member of the Infection Control Group in Azienda Ospedaliero-Universitaria Policlinico di Modena, Italy

Member of Italian Society of Infectious and Tropical Diseases (SIMIT)



**Synbiotics and their Potential in Maintaining Liver Health and Well Being**

**Dr. Alok Malaviya**

Industrial Biotechnologist, Founder and Director of QuaLife Biotech Pvt Ltd  
Associate Professor of Department of Life Sciences, Christ University, Bangalore, India



**Microfluidics Based Analytical Strategies and Approach for Microbial Detection**

**Professor Jörg P. Kutter**

Chairman of Analytical Biosciences at the Department of Pharmacy at the University of Copenhagen, Denmark.



**New EUCAST Breakpoints and Methods**

**Dr. Christian G. Giske**

Director of EUCAST  
Professor and Chief Consultant Physician Vice Chair of Committee for Research in Karolinska Institute  
Head of Division of Clinical Microbiology in Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden



**Phage Therapy Initiative in Finland**

**Professor Mikael Skurnik**

Research Director, Emeritus Professor of Bacteriology, Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki Researcher, Emeritus Head of Laboratory, Division of Clinical Microbiology, Helsinki University Hospital



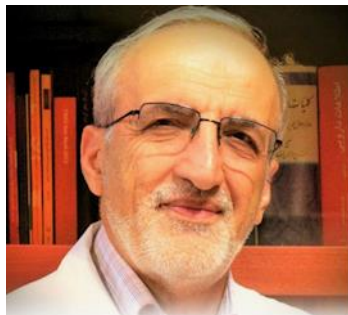
**A Novel Automated System for Malaria Diagnosis by using Artificial Intelligence Tools and a Universal Low-Cast Robotized Microscope**

**Dr. Joan Joseph Munne**

Assistant Physician of the Microbiology Service and Associate Researcher in Vall d'Hebron University Hospital, Spain



### Iranian Keynote Speakers



**Microbial Infection: A Major Etiology of  
Cancer**

**Professor Reza Malekzadeh**

Professor of Tehran University of Medical  
Sciences, Iran  
Adult Gastroenterology and Liver Specialist



**Novel Approaches in Controlling Chronic  
Non-Healing Wound Infections**

**Dr. Ali Pormohammad**

Post Doctoral Research Fellow, University of  
Calgary, Alberta, Canada  
Inventor and Innovator in the Field of Medical  
Microbiology



**Human Gut Microbiom and Disease  
Prediction**

**Dr. Azadeh Safarchi**

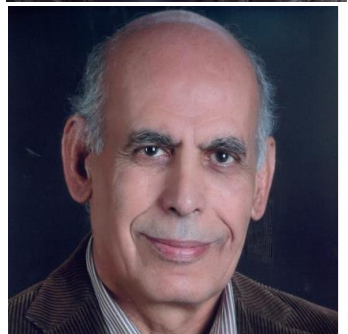
D.V.M, P.h.D  
Post-doctoral Reseach Fellow at CSIRO  
(Australia's Natiobal Science Agency)



**Introduction on Shotgun Metagenomics:  
From Sampling to Analysis**

**Dr. Jale Moradi**

Postdoctoral Researcher, Oral Health Center of  
Expertise, Norway



**Innovative Approach to Kala-azar  
Diagnosis, Treatment, and Prevention by  
Clinical Microbiology Research Center,  
Shiraz University of Medical Sciences**

**Professor Abdolvahab Alborzi**

Professor of Pediatric Infectious Diseases  
Founder of Clinical Microbiology Research  
Center  
Shiraz, Iran



**The role of non-antibiotic methods in the  
treatment of multi drug resistant  
microorganisms**

**Dr. Mohammadreza Salehi**

MD and IDS  
Research Center for Antibiotic Stewardship and  
Antimicrobial resistance  
Tehran University of Medical Science  
Tehran, Iran |P|PPS-



**Long COVID-19**

**Dr. Masoud Mardani**

Professor of Infectious Diseases  
Shahid Beheshti Medical University  
Tehran, Iran



**The position of microorganisms in the  
birth and evolution of life on Earth**

**Prof. Nasrin Moazami**

Microbial Biotechnologist, Iranian Research  
Organization for Science and Technology, Tehran  
(IROST)



**Proteomics and Mass Spectrometry**

**Dr. Sama Rezasoltani**

Postdoctoral Fellow of Alexander von Humboldt  
Foundation for Proteomics and Mass  
Spectrometry, Department of Clinical Chemistry  
and Laboratory Medicine, University Medical  
Center Hamburg-Eppendorf, Hamburg, Germany.



### Fundamental Microbiology and Microbial Biotechnology Speakers

<p><b>Dr. Akhavan Sepahi, Abbas</b> Associated Professor, Dep. Microbiology, Faculty of Biology, Islamic Azad University. Azad University.</p>	<p>The status of bioremediation in Iran and the countries of the Persian Gulf region</p>
<p><b>Dr. Behrad Vakylabad, Ali</b> Associate professor, Graduate university of advanced technology</p>	<p>Evolution of Copper Extraction from Low-grade Chalcopyrite Ores with Hybrid Bioleaching</p>
<p><b>Dr. Betesho Babrud, Ramsina</b> Nuclear Science and Technology Research Institute, Tehran, Iran</p>	<p>Radiation application in cultural heritage conservation</p>
<p><b>Dr. Dabbagh, Reza</b> Associate Professor, Nuclear Science and Technology Research Institute, Tehran, Iran</p>	<p>Bioremediation in nuclear industry by seaweeds biomass and cyanobacteria</p>
<p><b>Dr. Darezereshki, Esmaeel</b> Faculty of Engineering, Department of Materials Engineering, Shahid Bahonar University of Kerman, Kerman</p>	<p>Thermoacidophilic bioleaching of copper sulfide concentrate in the presence of chloride ions</p>
<p><b>Dr. Dastgheib, Mohammad Mehdi</b> Assistant Professor of Biotechnology, Research institute of petroleum industry (RIPI)</p>	<p>Applications of Biotechnology for Environmental Conservation in Iran (Experiences and Challenges)</p>
<p><b>Dr. Fooladi, Jamshid</b> Associated Professor, Director of Biotechnology Group Biological science faculty; Alzahra University</p>	<p>Probiotica: Yeast or Bacteria (advantages and disadvantages)</p>
<p><b>Dr. Habibi-Rezaei, Mehran</b> Professor in Biochemistry, University of Tehran</p>	<p>A perspective on enzyme mediated biotransformation and biorefining</p>
<p><b>Dr. Heidarieh, Marzieh</b> Associate Professor, Nuclear Science and Technology Research Institute, Tehran, Iran</p>	<p>Development of vaccine and synthesis of paraprobiotic using gamma irradiation</p>
<p><b>Dr. Javanmardi</b> Ph.D in Food Science and Technology</p>	<p>Post Biotic</p>
<p><b>Dr. Kargar, Mohammad</b> Professor of Microbiology Islamic Azad University, Shiraz Branch</p>	<p>New approaches biomining and recycling of Metals by using synthetic Biology</p>



<p><b>Dr. Lotfalian, Majid</b> Assistant professor, Graduate university of advanced technology</p>	<p>Chalcopyrite bioleaching, a sustainable rout for copper industries development</p>
<p><b>Dr. Majdi, Mohammad</b> Postdoctoral Research Associate, Miami University, USA Associate Professor, University of Kurdistan, Iran</p>	<p>Production of plant bioactive compounds in microbial systems via metabolic engineering and synthetic biology</p>
<p><b>Dr. Mehrshad, Malihe</b> Associate Professor in Mircobiology</p>	<p>Defining role of metabolic potential and interdependencies in sustaining the microbial community of deep groundwater ecosystems</p>
<p><b>Dr. Moghimi, Hamid</b> Associate professor in Microbiology, Department of Microbiology, School of Biology, University of Tehran</p>	<p>Bioremediation in extreme environments</p>
<p><b>Dr. Moradi, Homayoun</b> The chairman of the board of directors of the naturalist company</p>	<p>Where do we stand?</p>
<p><b>Dr. Nasr, Shaghayegh</b> Microorganisms Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran Department of Microbial Biotechnology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture</p>	<p>Probiotic Yeasts: biotechnological and functional characterization and their future potential</p>
<p><b>Dr. Nouri, Hoda</b> Biotechnology Biopharmaceutical business development supervisor BioPharma Business Deputy Behesta Daru</p>	<p>Yeast cell factories for biopharmaceuticals</p>
<p><b>Dr. Poorinmohammad, Naghmeh</b> Researcher and course lecturer at McGill University Microbial biotechnologist with expertise in microbial systems</p>	<p>Systems-level approaches for understanding and engineering of the oleaginous cell factory <i>Yarrowia lipolytica</i></p>
<p><b>Dr. Pooya, Mohammad</b> Assistant Professor, Molecular Biology Department, Pasteur Institute of Iran</p>	<p>“PUVA” treatment vs. bacteria”</p>
<p><b>Dr. Raouf Hosseini, Mohammad</b> Associate professor Department of Mining Engineering, Isfahan University of Technology, Isfahan, Iran</p>	<p>Heterotrophic bioleaching: Challenges and opportunities</p>





<p><b>Dr. Rezvani, Fariba</b> Assistant professor Research groups: Environmental and Industrial Biotechnology. Iranian Research Organization for Science and Technology (IROST)</p>	<p>Application of microalgae for removing organic substances and reducing dissolved solids from water and wastewater</p>
<p><b>Dr. Rostami, Khosrow</b> Assistant professor, Environmental and Industrial Biotechnology, Iranian Research Organization for Science and Technology</p>	<p>Potential of renewable sustainable energy to save the Planet</p>
<p><b>Dr. Saghfinia, Amir Asmaeil</b></p>	<p>Industrial Biotechnology: How to scale down the process</p>
<p><b>Dr. Shahbazi, Samira</b> Associate Professor, Nuclear Science and Technology Research Institute, Tehran, Iran</p>	<p>Gamma irradiation for induce mutation in microorganism to improve enzymatic metabolite production</p>
<p><b>Dr. Shahrokhi, Nader</b> Associate professor, Pasteur Institute of Iran</p>	<p>Antisense oligonucleotides, as promising antimicrobial Strategy</p>
<p><b>Dr. Shavandi, Mahmoud</b> Associate Professor in Microbiology. Biotechnology and Microbiology group, Biotechnology and Environment Research Division, Research institute of petroleum In dustry</p>	<p>Applications of Metagenomics in Petroleum Biotechnology</p>
<p><b>Dr. Soleimani, Reza</b> Prof. Sol Scientifics BV Gent, Belgium</p>	<p>New Beta-Lactamase Enzymes Specific Inhibitor for Treatent of Multi-Drug Resistant Bacteria</p>
<p><b>Dr. Tajer-Mohammad-Ghazvini, Parisa</b> Associate Professor, Nuclear Fuel Cycle Research School, Nuclear Science and Technology Research Institute, Tehran, Iran</p>	<p>Microbiological perspective on the interaction of microorganisms with uranium</p>
<p><b>Dr. Tefagh, Mojtaba</b> Blockchain Programme Manager at the University of Edinburgh, Scotland, UK Assistant Professor, Sharif University, Iran</p>	<p>Applications of integrative systems biology in the analysis of microbiome</p>
<p><b>Dr. Zamir, Seyed Morteza</b> Associate professor in Biochemical Engineering Faculty of Chemical Engineering Tarbiat Modares University</p>	<p>Sustainable treatment of industrial waste air using two-phase partitioning bioreactors and microbial composition shifts during the long-term operations</p>
<p><b>Dr. Zare-Mirakabad, Fatemeh</b> Assistant Professor, Amirkabir University of Technology, Iran</p>	<p>Using Machine Learning for Antibiotic Resistance Prediction</p>



## Congress Program- Medical Microbiology

**Monday 18 Sep**

Khwarizmi Hall

8:15-9	Opening Ceremony
9:00-9:20	<p><u>Welcome Speech</u></p> <p><b>Dr. Nasrin Moazami</b> (Microbial Biotechnologist)</p> <p><b>The position of microorganisms in the birth and evolution of life on Earth</b></p>
9:20-9:40	<p><b>Dr. Reza Malekzadeh</b> (Adult gastrointestinal and liver Specialist)</p> <p>Microbial Infections: One of the Main Causes of Cancer</p>
9:40-10	<p><b>Prof. Abdolvahab Alborzi</b> (Full Professor of Pediatric Infectious Diseases)</p> <p>Innovative Approach to Kala-azar Diagnosis, Treatment and Prevention by Clinical Microbiology Research Center, Shiraz University of Medical Science</p>
10-10:30	Coffee Break and Poster Viewing
10:30-12	<b>Panel on Laboratory Management</b>
<b>Panel Chair:</b>	<b>Dr. Hassan Morovvati</b> (DVM. Ph.D. of Anatomical Sciences) <b>Topic:</b> (Development and improvement of human resources in the laboratory)
<b>Panel Members:</b>	<p>Dr. Alireza Zarasvand (Director, Technology and innovation office, MSRT, IRAN) <b>Topic:</b> (Microbiology and Biotechnology situation in the technology and innovation ecosystem of Iran)</p> <p>Dr. Vahid Zare (Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization) <b>Topic:</b> (Strategic management in knowledge-based organizations)</p> <p>Dr. Gholamreza Hamzeloi (Head of Reference Health Lab of Tehran University of medical sciences) <b>Topic:</b> Quality assurance in clinical Microbiology laboratory</p> <p>Dr. Peyman Falsafi (Assistant Professor, Institute of Agricultural Education &amp; Extension, Agricultural Research, Education and Extension Organization, Tehran) <b>Topic:</b> (Knowledge management in education and research centers)</p> <p>Dr. Mohsen Lotfi (Associate Professor, Razi Vaccine &amp; Serum Research Institute, Agricultural Research, Education and Extension Organization, Karaj, Iran) <b>Topic:</b> (Good Laboratory Practices (GLP) principles in Research and Diagnostic Laboratories)</p> <p>Dr. Bizhan Nomanpour (Associate Prof. of Clinical Microbiology Microbiology Dept. Kermanshah University of Medical Sciences) <b>Topic:</b> (6 Sigma principles in clinical Microbiology laboratory)</p>
	<p><u>Keynote Speech</u></p> <p><b>Dr. Alireza Zarasvand</b> Director, Technology and innovation office, MSRT, IRAN</p> <p><b>Microbiology and Biotechnology situation in the technology and innovation ecosystem of Iran</b></p>
12-13:30	Prayer and Lunch Break



13:30-15	<b>Panel on the Antimicrobial Resistance</b>
<b>Panel Chair:</b>	<b>Dr. Kiarash Ghazvini</b> (Ph.D. of Microbiology) <b>Topic:</b> (Epidemiological trends of Vancomycin-resistant Enterococci isolated from healthcare-associated infections in Northeast of Iran during 2012 to 2022)
<b>Panel Members:</b>	Prof. Christian G. Giske (Professor, Karolinska Institute, Stockholm, Sweden) <b>Topic:</b> (New EUCAST Breakpoints and Methods) Dr. Ali Pormohammad (Postdoctoral Associate, Scientist and Medical Microbiologist, University of Alberta, Canada) <b>Topic:</b> (Novel Approaches in Controlling Chronic Non-Healing Wound Infections) Prof. Cristina Mussini (Professor, Director of the Infectious Diseases Clinics, University Hospital, Modena, Italy) <b>Topic:</b> (Strategies of Antimicrobial Stewardship) Dr. Mohammad Hassan Aelami (Pediatrician and Pediatric Infectious Specialist) <b>Topic:</b> (Antimicrobial resistance survey in university hospitals of Mashhad, Iran) Dr. Sepideh Hassanzadeh (Ph.D. of Bacteriology) <b>Topic:</b> (Exploring antibiotic usage and patterns of bacterial resistance)
<u>Keynote Speech</u>	
<b>Prof. Christian G. Giske</b> Director of EUCAST Professor and Chief Consultant Physician Vice Chair of Committee for Research in Karolinska Institute Head of Division of Clinical Microbiology in Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden	
<b>New EUCAST Breakpoints and Methods</b>	
15:00-15:30	Coffee Break and Poster Viewing
15:30-17:00	<b>Panel on the Infection Prevention and Control</b>
<b>Panel Chair:</b>	<b>Dr. Arash Seifi</b> (Infectious Diseases Specialist) <b>Topic:</b> (Epidemiology of Healthcare associated infections)
<b>Panel Members:</b>	Dr. Marianna Maschiari (Infectious and Tropical Diseases Specialist, Italy) <b>Topic:</b> (Strategies of Infectious Control) Dr. Davood Yadegarinia (Infectious and Tropical Diseases Specialist, Shahid Beheshti University of Medical Sciences) <b>Topic:</b> (Introduction) Dr. Esmail Mohammadnejad (Ph.D. of Nursing, Head of Infection Control at Imam Khomeini Hospital Complex) <b>Topic:</b> (Prevention and Control of MDR Organisms in ICUs) Dr. Fahimeh Hadavand (Infectious and Tropical Diseases Specialist) <b>Topic:</b> (MRSA: Prevention of transmission in hospitals) Dr. Hamid Solgi (Ph.D. of Bacteriology) <b>Topic:</b> (Successful Control of outbreak)
<u>Keynote Speech</u>	
<b>Dr. Marianna Maschiari</b> Coordinator of Antimicrobial Stewardship Group and Active Member of the Infection Control Group in Azienda Ospedaliero-Universitaria Policlinico di Modena, Italy Member of Italian Society of Infectious and Tropical Diseases (SIMIT) <b>Strategies of Infectious Control</b>	



Abooreyhan Hall

10:30-12	<b>Panel on the Role of Microorganisms in Cancer</b>
<b>Panel Chair:</b>	<b>Dr. Masoud Alebouyeh</b> (Ph.D. of Bacteriology) <b>Topic:</b> (New findings in Helicobacter pylori infection and its role in the gastric cancer)
<b>Panel Members:</b>	Prof. Hartmut Schlüter (Professor of University Medical Center Hamburg-Eppendorf, Member of the Board of Directors of the Consortium for Top-Down Proteomics (CTDP), Head of Section "Mass Spectrometric Proteomics" of the UKE) <b>Topic:</b> (Analysis of Proteoforms – closer to the relationship of proteins and their functions) Dr. Seyed Reza Mohebbi (Medical Virologist) <b>Topic:</b> (Hepatitis B virus and Hepatocellular carcinoma; an insight into molecular mechanisms of HBV carcinogenesis) Dr. Saeid Latifi-Navid (Medical Molecular Genetics) <b>Topic:</b> (Helicobacter pylori, Host Genetics, Gastric Microbiota and Gastric Cancer Risk) Dr. Omid Memarsadeghi (MD, PhD, Dermatologist) <b>Topic:</b> (Microbiome of Skin Cancer) Prof. Hartmut Schlüter (Professor of University Medical Center Hamburg-Eppendorf, Member of the Board of Directors of the Consortium for Top-Down Proteomics (CTDP), Head of Section "Mass Spectrometric Proteomics" of the UKE) <b>Topic:</b> (Analysis of Proteoforms – closer to the relationship of proteins and their functions)
<u>Keynote Speech</u>	
<b>Prof. Hartmut Schlüter</b> Professor of University Medical Center Hamburg-Eppendorf, Member of the Board of Directors of the Consortium for Top-Down Proteomics (CTDP), Head of Section "Mass Spectrometric Proteomics" of the UKE <b>Analysis of Proteoforms – closer to the relationship of proteins and their functions</b>	
12-13:30	Prayer and Lunch Break
13:30-15	<b>Panel on the Nanomicrobiology: The role of Microorganisms in Nanotechnology</b>
<b>Panel Chair:</b>	<b>Prof. Seyed Mahdi Rezayat</b> (Professor of Pharmacology, Tehran University of Medical Sciences)
<b>Panel Members:</b>	Dr. Fatemeh Gheybi (Medical Nanotechnology) <b>Topic:</b> (Application of nano in microbiology) Professor Jörg P. Kutter (Chairman of Analytical Biosciences at the Department of Pharmacy at the University of Copenhagen, Denmark) <b>Topic:</b> (Microfluidics Based Analytical Strategies and Approach for Microbial Detection) Prof. Bitra Mehravi (Professor of Medical Nanotechnology) <b>Topic:</b> (Application of photocatalytic-based nanostructured materials disinfection) Dr. Somayeh Daneshjoo (Ph.D. of Nanobiotechnology) <b>Topic:</b> (Study of biosynthesized nickel nanoparticles and antimicrobial activities from bacillus megaterium) Dr. Masoomeh Amini (Ph.D. of Bacteriology) <b>Topic:</b> (B-glucan and its liposomal form as effective antibiotic alternative in poultry) Dr. Hamid Pajavand (Ph.D. of Bacteriology) <b>Topic:</b> (Synthesis of CDs by hydrothermal method and evaluation of its antibacterial and anti-biofilm effect against antibiotic-resistant S. aureus and P. aeruginosa strains)
<u>Keynote Speech</u>	
<b>Professor Jörg P. Kutter</b> Chairman of Analytical Biosciences at the Department of Pharmacy at the University of Copenhagen, Denmark <b>Microfluidics Based Analytical Strategies and Approach for Microbial Detection</b>	
15:00-15:30	Coffee Break and Poster Viewing



15:30-17:00	<b>Panel On Metagenomics</b>
<b>Panel Chair:</b>	<b>Dr. Maryam Eslami</b> (MD., Ph.D. in Genetics, and Regenerative Medicine Fellowship)
<b>Panel Members:</b>	<p>Dr. Maria Riedner (Coordinator of the Technology Platform Mass Spectrometry University of Hamburg, Hartmut Schlüter's Lab, Hamburg, Germany) <b>Topic:</b> (Tissue sampling with infrared-laser systems – getting closer to the original composition of molecules in tissues)</p> <p>Dr. Azadeh Safarchi (Ph.D. of Microbiology, Postdoctoral Research Fellow at CSIRO, Australia) <b>Topic:</b> (Human Gut Microbiom and Disease Prediction)</p> <p>Dr. Jale Moradi (Ph.D. of Bacteriology, Postdoctoral Researcher, Oral Health Center of Expertise, Norway) <b>Topic:</b> (Introduction on Shotgun Metagenomics: From Sampling to Analysis)</p> <p>Dr. Sama Rezasoltani (Ph.D. of Microbiology, Postdoctoral Fellow of Alexander von Humboldt Foundation, Hamburg, Germany) <b>Topic:</b> (Proteomics and Mass Spectrometry)</p> <p>Dr. Mohammadali Khosravi (Ph.D of Biotechnology) <b>Topic:</b> (CRISPR revolution: unlocking the potential of microbial genomes for Biotechnology and beyond)</p>
<p><u>Keynote Speech</u></p> <p><b>Dr. Maria Riedner</b> Coordinator of the Technology Platform Mass Spectrometry University of Hamburg, Hartmut Schlüter's Lab, Hamburg, Germany <b>Tissue sampling with infrared-laser systems – getting closer to the original composition of molecules in tissues</b></p>	

Andisheh Hall

13:30-15	<b>Oral Presentation: (Young Researchers)</b>
<b>Panel Chair:</b>	<b>Dr. Morvarid Shafiei</b> <b>Topic:</b> (Bacteriophage-Delivering Hydrogel offers simultaneous promise against methicillin-resistant Staphylococcus aureus (MRSA) causing bedsore and diabetic wounds)
<b>Panel Members:</b>	Golnar Rahimzadeh, Shahin Sheiki, Zohre Eshaghi Seijani, Somayeh Kermani, Hajar Hajian, Hamidreza Gelyan, Batool Kaviani, Abbas Zarei, Zenab Ghanei Aghmooyei



**Tuesday 19 Sep**

Abooreyhan Hall

8:30-10:00	<b>Panel on the Prebiotics and Probiotics</b>
<b>Panel Chair:</b>	<b>Dr. Saeed Mirdamadi</b> (Ph.D. of Biotechnology) <b>Topic:</b> (Probiotics and their bioactive derivatives, opportunities and challenges)
<b>Panel Members:</b>	Prof. Hartmut Schlüter (Professor of University Medical Center Hamburg-Eppendorf, Member of the Board of Directors of the Consortium for Top-Down Proteomics (CTDP), Head of Section "Mass Spectrometric Proteomics" of the UKE) <b>Topic:</b> (Analysis of Proteoforms – closer to the relationship of proteins and their functions) Dr. Omid Pajand (Ph.D. of Medical Microbiology) <b>Topic:</b> (Effects of probiotic supplementation on exercise; Can probiotics make our muscle stronger?) Dr. Parvaneh Jafari (Ph.D. of Microbiology) Dr. Marzieh Hosseininezhad (Ph.D. of Food Microbiology) <b>Topic:</b> (Progressive concept of postbiotic metabolites, new horizons of applied microbiology in functional foods and nutraceuticals) Dr. Mahdi Rohani (Ph.D. of Medical Bacteriology) <b>Topic:</b> (Evaluation of native probiotic bacteria on gut inflammation status via different signaling pathways)
<u>Keynote Speech</u>	
<b>Prof. Hartmut Schlüter</b> Professor of University Medical Center Hamburg-Eppendorf, Member of the Board of Directors of the Consortium for Top-Down Proteomics (CTDP), Head of Section "Mass Spectrometric Proteomics" of the UKE <b>Analysis of Proteoforms – closer to the relationship of proteins and their functions</b>	
10:00-10:30	Coffee Break and Poster Viewing
10:30-12:00	<b>Panel on Bacteriophages and Phage Therapy</b>
<b>Panel Chair:</b>	<b>Dr. Nour Amirmozafari</b> (Ph.D. of Microbiology)
<b>Panel Members:</b>	Prof. Mikael Skurnik (Emeritus Professor of Bacteriology, Helsinki, Finland) <b>Topic:</b> (Phage Therapy Initiative in Finland) Dr. Raheleh Majdani (Ph.D. of Microbiology) <b>Topic:</b> (phage therapy: challenges and opportunities) Dr. Shakiba Darvish Alipoor (Ph.D. of Microbiology) <b>Topic:</b> (Comparision of antibacterial activity of bacteriophage and phage-derived endolysin) Dr. Golnar Rahimzadeh (Ph.D. of Microbiology) <b>Topic:</b> (Phage therapy to preventing bacterial pneumonia in patients: A double-blind clinical trial study)
<u>Keynote Speech</u>	
<b>Prof. Mikael Skurnik</b> Research Director, Emeritus Professor of Bacteriology, Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki Researcher, Emeritus Head of Laboratory, Division of Clinical Microbiology, Helsinki University Hospital, Finland <b>Phage Therapy Initiative in Finland</b>	
12-13:30	Prayer and Lunch Break



13:30-15	<b>Panel on the Microbiology and Pharmacology</b>
<b>Panel Chair:</b>	<b>Prof. Arash Mahboubi</b> (Professor of Pharmaceutics)
<b>Panel Members:</b>	Dr. Nasrin Samadi (Ph.D. of Pharmaceutics) Dr. Hossein Jamalifar (Ph.D. of Microbiology) Prof. Hamidreza Moghimi (Professor of Pharmaceutics) Parastoo Rezaee (Ph.D. of Microbiology, Head of Quality Control of Varian Pharmed Pharmaceutical Company)

Andisheh Hall

8:30-10:00	<b>Panel on the Tuberculosis</b>
<b>Panel Chair:</b>	<b>Dr. Abbas Ali Imani</b> Fooladi (Professor of Medical Bacteriology) Panel Members: Dr. Jalil Kardan (Quality Control and Screening Management Office, Deputy of Technical and New Technologies, Iranian Blood Transfusion Organization) <b>Topic:</b> (Mycobacterium; Health and Disease)
<b>Panel Members:</b>	Dr. Mohammad Javad Nasiri (Ph.D. of Microbiology) Dr. Mostafa Ghalami Nobar (MSc of Microbiology) Dr. Sirus Amini (Ph.D. of Microbiology) Dr. Hossein Kazemian (Assistant Professor, Department of Medical Microbiology, Ilam University of Medical Science) <b>Topic:</b> The growing challenge of Non-Tuberculosis Mycobacteria
10:00-10:30	Coffee Break and Poster Viewing
10:30-12:00	<b>Oral presentations: (Young Researcher)</b>
<b>Panel Chair:</b>	<b>Dr. Mehri Haeili</b> <b>Topic:</b> (Multifactorial resistance mechanisms associated with tigecycline resistance in Escherichia coli)
<b>Panel Members:</b>	Masoumeh Rajabzadeh, Mohammad Milad Shirandehi, Kambiz Feizi, Davood Javanmard, Parya Barooni Reshno, Faezeh Enbe Ali, FARiba Mofidi, Mina Shirmohammadpour, Samaneh Kheibari
15:00-15:30	Coffee Break and Poster Viewing
15:30-17:00	<b>Oral presentations: (Young Researcher)</b>
<b>Panel Chair:</b>	Dr. Naseh Maleki <b>Topic:</b> (Microbial profiling of <i>Paederus fuscipes</i> (Coleoptera: Staphylinidae) through Next-Generation Sequencing
<b>Panel Members:</b>	Sepideh Soltani, Dalileh Aslani, Alireza Hatami Rad, Ahad Shahmaleki, Afsaneh Mola Mirzaei, Hadiseh Rabieci, Fatemeh Beh Oftadeh, Zahra Tahmasebi, Mina Latifian, Zahra Davtalab Toosi



**Wednesday 20 Sep**

Abooreyhan Hall

8:30-10:00	<b>Panel on the Emerging and Reemerging Diseases</b>
<b>Panel Chair:</b>	<b>Prof. Ehsan Mostafavi</b> (Professor of Epidemiology) <b>Topic:</b> (What will the future pandemics look like and how prepared are we to deal with them?)
<b>Panel Members:</b>	Dr. Masoud Mardani (Professor of Infectious Disease, Shahid Beheshti Medical University) <b>Topic:</b> (Long COVID-19) Dr. Ahmad Raeisi (Communicable Diseases Management Centre) <b>Topic:</b> (An overview of the latest malaria situation in Iran) Dr. Seyed Mohsen Zahraei (Infectious and Tropical Diseases Specialist, Head of the Vaccine-Preventable Diseases Department, Communicable Diseases Management Centre) <b>Topic:</b> (Elimination of measles and rubella and the challenges of maintaining it) Dr. Saber Esmaeili (Ph.D. of Medical Bacteriology, Pasteur Institute of Iran) <b>Topic:</b> (Identification of tick-borne rickettsia in Iran) Dr. Abdolreza Mir-Oliaei (Communicable Diseases Management Centre) <b>Topic:</b> (Aedes mosquito in Iran, an emerging threat that needs attention) Dr. Fahmieh Bagheri Amiri (Ph.D of Epidemiology, Pasteur Institute of Iran) <b>Topic:</b> (Microbial resistance in Iran, a serious threat but less attention) Dr. Neda Baseri (Ph.D. in Medical Bacteriology; Pasteur Institute of Iran) <b>Topic:</b> (Q fever, an emerging disease with public health importance in Iran)
<u>Keynote Speech</u>	
<b>Dr. Masoud Mardani</b> Professor of Infectious Disease, Shahid Beheshti medical University, MD, MPH <b>Long Covid 19</b>	
10:00-10:30	Coffee Break and Poster Viewing
10:30-12	<b>Panel on Infection in Immunocompromised Patients</b>
<b>Panel Chair:</b>	<b>Dr. Sara Abolghasemi</b> (Infectious and Tropical Diseases Specialist, Immunocompromised Host Fellowship) <b>Topic:</b> (Management of CMV infection in immunocompromised patients)
<b>Panel Members:</b>	Dr. Mohammadreza Salehi (MD and IDS, Research Center for Antibiotic Stewardship and Antimicrobial resistance, Tehran University of Medical Sciences) <b>Topic:</b> (The role of non-antibiotic methods in the treatment of multi drug resistant microorganisms) Prof. Stefania Stefani (Professor of Microbiology and Medical Microbiology, University of Catania, Italy) <b>Topic:</b> (The Importance of Fast Microbiology in the Antimicrobial stewardship era) Prof. Seyed Alireza Naji (Professor of Medical Virology, Virology Research Center, Dr. Masih Deneshvari Hospital) <b>Topic:</b> (How to manage drug-resistant CMV infections?) Dr. Zahra Abtahian (Infectious and Tropical Diseases Specialist, Immunodeficiency Fellowship, National Research Institute of Tuberculosis and Lung Diseases, Dr. Masih Daneshvari Hospital.) <b>Topic:</b> (Management of Pneumonia of immunocompromised patients) Dr. Hamed Fakhim (Assistant Professor of Medical Mycology, Infectious Diseases and Tropical Medicine Research Center) <b>Topic:</b> (How to manage antifungal resistant infections in immunocompromised patients?) Dr. Elaheh Nasri (Infectious and Tropical Diseases Specialist, Immunodeficiency Fellowship) <b>Topic:</b> (Management of pneumocystis jirovici pneumonia in immunocompromised patients)
<u>Keynote Speech</u>	
<b>Dr. Mohammadreza Salehi</b> MD and IDS, Research Center for Antibiotic Stewardship and Antimicrobial resistance, Tehran University of Medical Science <b>The role of non-antibiotic methods in the treatment of multi drug resistant microorganisms</b>	
12:00-12:30	Closing Ceremony





## Congress Program- The General Microbiology and Microbial Biotechnology

**Monday 18 Sep**

### Khwarizmi Hall

8:15-9	Opening Ceremony
9:00-9:20	<u>Welcome Speech</u> <b>Dr. Nasrin Moazami</b> (Microbial Biotechnologist) <b>The position of microorganisms in the birth and evolution of life on Earth</b>
9:20-9:40	<b>Dr. Reza Malekzadeh</b> (Adult gastrointestinal and liver Specialist) Microbial Infections: One of the Main Causes of Cancer
9:40-10	<b>Prof. Abdolvahab Alborzi</b> (Full Professor of Pediatric Infectious Diseases) Innovative Approach to Kala-azar Diagnosis, Treatment and Prevention by Clinical Microbiology Research Center, Shiraz University of Medical Science
10-10:30	Coffee Break and Poster Viewing

### Farabi Hall

10:30-12	<b>Panel on the Role of Genetics of Microorganisms and Molecular Biotechnology</b>
<b>Panel Chair:</b>	<b>Dr. Hamideh Ofoghi</b> (Associate professor of Molecular Biotechnology at Department of Biotechnology, Iranian Research Organization for Science and Technology (IROST))
<b>Panel Members:</b>	Dr. Reza Soleimani (Sol Scientifics BV Gent, Belgium) Dr. Nader Shahrokhi (Associate professor/Pasteur Institute of Iran) Dr. Mohammad Pooya (Assistant Professor, Molecular Biology Department, Pasteur Institute of Iran) Dr. Soheila Moradi Bidhendi (Microbiologist, Associate Professor Department of Microbiology Reference Laboratory of Leptospira & Salmonella Razi Vaccine & Serum Research Institute Karaj, Iran)
	<u>Keynote Speech</u> <b>Prof. Reza Soleimani</b> Prof. Sol Scientifics BVGent Belgium <b>New Beta-Lactamase Enzymes Specific Inhibitor for Treatment of Multi-Drug Resistant Bacteria</b> <b>Dr. Nader Shahrokhi</b> Pasteur Institute of Iran / Associate professor <b>Antisense oligonucleotides, as promising antimicrobial Strategy</b> <b>Dr. Mohammad Pooya</b> Assistant Professor, Molecular Biology Department, Pasteur Institute of Iran <b>"PUVA" treatment vs. bacteria"</b>
12-13:30	Prayer and Lunch Break



13:30-15	<b>Panel on the Convergence of Biotechnology with Nuclear Science</b>
<b>Panel Chair:</b>	<b>Dr. Parisa Tajer-Mohammad-Ghazvini</b> (Associate Professor, Nuclear Fuel Cycle Research School, Nuclear Science and Technology Research Institute, Tehran, Iran)
<b>Panel Members:</b>	Dr. Reza Dabbagh (Associate Professor, Nuclear Science and Technology Research Institute, Tehran, Iran) Dr. Ramsina Betesho Babrud (Nuclear Science and Technology Research Institute, Tehran, Iran) Dr. Marzieh Heidarieh (Associate Professor, Nuclear Science and Technology Research Institute, Tehran, Iran) Dr. Samira Shahbazi (Associate Professor, Nuclear Science and Technology Research Institute, Tehran, Iran)
<u>Keynote Speech</u>	
<p><b>Dr. Parisa Tajer-Mohammad- Ghazvini</b> Associate Professor, Nuclear Fuel Cycle Research School, Nuclear Science and Technology Research Institute, Tehran, Iran</p> <p><b>Microbiological perspective on the interaction of microorganisms with uranium</b></p>	
<p><b>Dr. Reza Dabbagh</b> Associate Professor, Nuclear Science and Technology Research Institute, Tehran, Iran</p> <p><b>Bioremediation in nuclear industry by seaweeds biomass and cyanobacteria</b></p>	
<p><b>Dr. Ramsina Betesho Babrud</b> Nuclear Science and Technology Research Institute, Tehran, Iran</p> <p><b>Radiation application in cultural heritage conservation</b></p>	
<p><b>Dr. Marzieh Heidarieh</b> Associate Professor, Nuclear Science and Technology Research Institute, Tehran, Iran</p> <p><b>Development of vaccine and synthesis of paraprobiotic using gamma irradiation</b></p>	
<p><b>Dr. Samira Shahbazi</b> Associate Professor, Nuclear Science and Technology Research Institute, Tehran, Iran</p> <p><b>Gamma irradiation for induce mutation in microorganism to improve enzymatic metabolite production</b></p>	
15:00-15:30	Coffee Break and Poster Viewing
15:30-17:00	<b>Panel on The Extremophiles and biotechnology - Microbial Biodiversity and Culture Collections</b>
<b>Panel Chair:</b>	<b>Dr. Farzaneh Azizmohseni</b> (Head of Industrial Microorganisms Collection Center, (Persian Type Culture Collection (PTCC)) at Iranian Research Organization for Science and Technology (IROST).)
<b>Panel Members:</b>	Dr. Bita Asgari (Department of Botany, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran) Dr. Philippe Desmeth (Belgian Science Policy Office Belgian Coordinated Collections of Micro-organisms WTC III Simon Bolivar Boulevard, 30 - 1000 Brussels, Belgium)
<u>Keynote Speech</u>	
<p><b>Dr. Bita Asgari</b> (Department of Botany, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran)</p> <p><b>Fungal Contributions to Sustainable Agriculture and the Challenges in Biodiversity Conservation</b></p>	
<p><b>Dr. Philippe Desmeth</b> Belgian Science Policy Office Belgian Coordinated Collections of Micro-organisms WTC III Simon Bolivar Boulevard, 30 - 1000 Brussels, Belgium</p> <p><b>Biobanks for "Bio-vention"</b></p> <p><b>Culture Collections, professionals underpinning microbial resources exploitation.</b></p>	



**Tuesday 19 Sep**

Farabi Hall

8:30-10:00	<b>Panel on the Microbial biomass production</b>
<b>Panel Chair:</b>	<b>Dr. Maryam Tajabadi Ebrahimi</b> (Associate Professor in Microbiology, Department of Biology, Science faculty, IAU, Central Tehran Branch.CEO of Takgene Group (Nova gene, Lactovgene, Bogan, Asugene, Zist Mahsool Parsian))
<b>Panel Members:</b>	Dr. Jamshid Fooladi Probiotica (Associated Professor, Director of Biotechnology Group Biological science faculty; Alzahra University) Dr. Moradi (The chairman of the board of directors of the naturalist company)
<p><u>Keynote Speech</u></p> <p><b>Dr. Jamshid Fooladi</b> Associated Professor, Director of Biotechnology Group Biological science faculty; Alzahra University <b>Probiotica: Yeast or Bacteria (advantages and disadvantages)</b></p> <p><b>Dr. Homayoun Moradi</b> The chairman of the board of directors of the naturalist company <b>Where do we stand?</b></p> <p><b>Dr. Javanmardi</b> Ph.D. in Food Science and Technology <b>Post Biotic</b></p>	
10:00-10:30	Coffee Break and Poster Viewing
10:30-12:00	<b>Panel on The Bioinformatics, Systems Biology and Synthetic Biology of Microorganisms</b>
<b>Panel Chair:</b>	<b>Dr. Farshad Darvishi</b> (Professor of Microbiology, Alzahra University and Researcher of Synthetic Biology and Metabolic Engineering, Technical University of Denmark)
<b>Panel Members:</b>	Dr. Mohammad Majdi (Postdoctoral Research Associate, Miami University, USA Associate Professor, University of Kurdistan, Iran) Dr. Mojtaba Tefagh (Assistant Professor, Sharif University, Iran, Blockchain Programme Manager at the University of Edinburgh, Scotland, UK) Dr. Fatemeh Zare-Mirakabad (Assistant Professor, Amir Kabir University of Technology, Iran)
<p><u>Keynote Speech</u></p> <p><b>Dr. Mojtaba Tefagh</b> Assistant Professor, Sharif University, Iran, Blockchain Programme Manager at the University of Edinburgh, Scotland, UK <b>Applications of integrative systems biology in the analysis of microbiome</b></p> <p><b>Dr. Mohammad Majdi</b> Postdoctoral Research Associate, Miami University, US, Associate Professor, University of Kurdistan, <b>Production of plant bioactive compounds in microbial systems via metabolic engineering and synthetic biology</b></p> <p><b>Dr. Fatemeh Zare-Mirakabad</b> Assistant Professor, Amirkabir University of Technology <b>Using Machine Learning for Antibiotic Resistance Prediction</b></p>	



12-13:30	Prayer and Lunch Break
13:30-15	<b>Panel on the Microbial Biochemistry, Enzyme Technology, and Protein Engineering and Bioactive Compounds</b>
<b>Panel Chair:</b>	<b>Dr. Mohammad Reza Soudi</b> (Professor of Microbiology Department of Microbiology, Faculty of Biological Sciences Alzahra University. Manager of Sib Zist Fan, a knowledge-based Company.)
<b>Panel Members:</b>	Dr. Naghmeh Poorinmohammad (Researcher and course lecturer at McGill University Microbial biotechnologist with expertise in microbial systems)
<u>Keynote Speech</u>	
<b>Dr. Naghmeh Poorinmohammad</b> Researcher and course lecturer at McGill University Microbial biotechnologist with expertise in microbial systems <b>Systems-level approaches for understanding and engineering of the oleaginous cell factory <i>Yarrowia lipolytica</i></b>	
15:00-15:30	Coffee Break and Poster Viewing
15:30-17:00	<b>Panel on The Fungal Biotechnology</b>
<b>Panel Chair:</b>	<b>Dr. Hamid Moghimi</b> (Assistant Professor in Microbiology, Department of Microbiology, University of Tehran.)
<b>Panel Members:</b>	Dr. Hoda Nouri (Ph.D. in Microbial Biotechnology Biopharmaceutical business development supervisor, BioPharma Business Deputy Behesta Daru) Dr. Shaghayegh Nasr (Department of Microbial Biotechnology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran)
<u>Keynote Speech</u>	
<b>Dr. Hoda Nouri</b> Ph.D. in Microbial Biotechnology Biopharmaceutical business development supervisor, BioPharma Business Deputy Behesta Daru <b>yeast cell factories for biopharmaceuticals</b>	
<b>Dr. Shaghayegh Nasr</b> Department of Microbial Biotechnology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture <b>Probiotic Yeasts: biotechnological and functional characterization and their future potential</b>	
<b>Dr. Hamid Moghimi</b> Associate professor in microbiology, Department of microbiology, School of biology, University of Tehran <b>Bioremediation in extreme environments</b>	

Andisheh1 Hall

8:30-10:00	<b>Panel on The Microbiology of cultural heritage</b>
<b>Panel Chair:</b>	<b>Dr. Parisa Mohammadi</b> (Associate Professor of Microbiology Department of Microbiology, Faculty of Biological Sciences Alzahra University. Former president of Cultural Heritage and Tourism Research Institute.)
<b>Panel Members:</b>	Dr. Hamed Vahdati Nasab (Professor, Department of Archaeology, Tarbiat Modares University) Dr. Atoosa Momeni (Archaeologist, Head of Intangible Heritage Center) Dr. Atefe Shekofte (Ph.D. Senior Scientific Researcher, Metropolitan Museum of Art, NY, USA) Dr. Azam Aliasgari Veshareh (Postdoc Researcher, Research Center for Applied Microbiology and Microbial Biotechnology, Alzahra University) Dr. Leila Shokrzadeh (Assistant Professor, Shandiz Institute of Higher Education, Mashhad, Iran) Dr. Negar Raesnia (PhD in Microbiology, Department of Restoration and Preservation, National Library and Archives of Iran) Maryam Ijadpanahsaravi (Researcher, Department of Biology, Utrecht University, Netherlands)



Keynote Speech

**Dr. Hamed Vahdati Nasab**

Department of Microbiology, Alzahra University

**Effect of Neanderthal's Genes in Contemporary Humans Diseases**

**Dr. Atefe Shekofteh**

Metropolitan Museum of Art, NY, USA

**Biom mineralization Function in Conservation of Calcareous Stone of Built Heritage: A perspective to the Further Investigations**

**Maryam Ijadpanahsaravi**

Department of Biology, Utrecht University, Netherlands

**The Silent Threat: Aspergillus Species Spores and Biodeterioration of Cultural Heritage**

**Dr. Negar Raeisnia**

Department of Restoration and Preservation, National Library and Archives of Iran

**Biodeterioration of Archival and Library Materials: Prevention and Control**

**Dr. Leila Shokrzadeh**

Shandiz Institute of Higher Education, Mashhad, Iran

**Different Methods for Diagnosis of Microbial Deterioration of Cultural Heritage**

10:00-10:30

Coffee Break and Poster Viewing

10:30-12:00

**Panel on the Geomicrobiology and Biohydrometallurgy**

**Panel  
Chair:**

**Dr. Zahra Manafi** (Head of Hydrometallurgy Unit in Research and Development Department, Sarcheshmeh Copper Complex PhD in Microbiology.)

**Panel  
Members:**

Dr. Mohammad Kargar (Professor of Microbiology Islamic Azad University, Shiraz Branch)  
Dr. Majid Lotfalian (Assistant professor, Graduate university of advanced technology)  
Dr. Mohammad Raouf Hosseini (Associate professor, Department of Mining Engineering, Isfahan University of Technology)  
Dr. Esmaeel Darezereshki (Faculty of Engineering, Department of Materials Engineering, Shahid Bahonar University of Kerman)  
Dr. Ali Behrad Vakylabad (Associate professor, Graduate university of advanced technology)

Keynote Speech

**Pro. Mohammad Kargar**

(Professor of Microbiology Islamic Azad University, Shiraz Branch)

**New approaches biomining and recycling of Metals by using synthetic Biology**

**Dr. Majid Lotfalian**

Assistant professor, Graduate university of advanced technology

**Chalcopyrite bioleaching, a sustainable rout for copper industries development**

**Dr. Mohammad Raouf Hosseini**

Associate professor, Department of Mining Engineering, Isfahan University of Technology

**Heterotrophic bioleaching: Challenges and opportunities**

**Dr. Esmaeel Darezereshki**

Faculty of Engineering, Department of Materials Engineering, Shahid Bahonar University of Kerman

**Thermoacidophilic bioleaching of copper sulfide concentrate in the presence of chloride ions**

**Dr. Ali Behrad Vakylabad**

Associate professor, Graduate university of advanced technology

**Evolution of Copper Extraction from Low-grade Chalcopyrite Ores with Hybrid Bioleaching**



**Wednesday 20 Sep**

Farabi Hall

8:30-10:00	<b>Panel on the Biochemical engineering and microbial processes and Bio-fuels and Bio-based materials</b>
<b>Panel Chair:</b>	<b>Dr. Mohsen Nosrati</b> (Associate Professor, Biotechnology Group, Department of Chemical Engineering, Tarbiat Modarres University) <b>Dr. Hossein Ghanavati</b> (Associate Professor and Head of Microbial Biotechnology Group, Agricultural Biotechnology Research Institute of Iran (ABRII))
<b>Panel Members:</b>	Prof. Mohammad Pazouki (Professor, Research Department of Energy, Materials and Energy research Center) Dr. Khosrow Rostami (Assistant professor, Environmental and Industrial Biotechnology, Iranian Research Organization for Science and Technology (IROST)) Dr. Homa Torabizadeh (Assistant Professor in Food Science & Technology Iranian Research Organization for Science and Technology (IROST)) Dr. Mehrdad Azin (Professor of Industrial Microbiology, Iranian Research Organization for Science and Technology (IROST))
<u>Keynote Speech</u>	
<b>Dr. Seyed Morteza Zamir</b> Associate Professor at the Faculty of Chemical Engineering, Tarbiat Modares University <b>Two-phase-distributed bioreactors for sustainable treatment of polluted industrial air</b>	
<b>Dr. Khosrow Rostami</b> Assistant professor, Environmental and Industrial Biotechnology, Iranian Research Organization for Science and Technology <b>Potential of renewable sustainable energy to save the Planet</b>	
<b>Dr.Homa Torabizadeh</b> Associate Prof. of Chemical Industries Institute Department of Food Science & Technology <b>Multi-immobilization of Cabohydrases and Proteases by Nanomagnetic Combi-CLEAs Method for Oil and Protein Hydrolysates Extraction from Oil Seeds in Aqueous Phase</b>	
10:00-10:30	Coffee Break and Poster Viewing
10:30-12	<b>Panel on the microbiology of water, air and environment</b>
<b>Panel Chair:</b>	<b>Dr. Abbas Farazmand</b> (Assistant Professor, Environmental and Industrial Biotechnology. Iranian Research Organization for Science and Technology (IROST))
<b>Panel Members:</b>	Dr. Mohammad Mehdi Dastgheib (Associated Professor of Biotechnology, Research Institute of Petroleum Industry (RIPI), Tehran, Iran) Dr. Soheila Shokrollahzadeh (Professor in Chemical Engineering Iranian Research Organization for Science and Technology (IROST)) Dr. Fariba Rezvani (Assistant professor, Environmental and Industrial Biotechnology. Iranian Research Organization for Science and Technology (IROST))
<u>Keynote Speech</u>	
<b>Dr. Mohammad Mehdi Dastgheib</b> Associated Professor of Biotechnology, Research institute of petroleum industry (RIPI), Tehran, Iran <b>Applications of Biotechnology for Environmental Conservation in Iran (Experiences and Challenges)</b>	
<b>Dr. Fariba Rezvani</b> Assistant professor, Environmental and Industrial Biotechnology. Iranian Research Organization for Science and Technology (IROST) <b>Application of microalgae for removing organic substances and reducing dissolved solids from water and wastewater</b>	
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**24<sup>th</sup> IRAN'S INTERNATIONAL  
CONGRESS OF MICROBIOLOGY**



Iranian Society of  
Microbiology

# Panel Member Lectures



## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# A Novel System Integrating Electrolysis and Ionic Membranes (EIMs) Enables the Artificial Carbon Concentration and Alleviation of Metal Cation Stress in Microalgae Cultivation

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## ABSTRACT

Microalgae-based carbon sequestration is emerging as a green and sustainable way to achieve negative carbon while recycling CO<sub>2</sub> into biomass used for the production of bioenergy and value-added products. However, its successful implementation is still to be realized due to the low solubility of CO<sub>2</sub> and ion accumulation with the addition of bicarbonate in the culture medium. In this study, we proposed, developed and verified a novel system integrating electrolysis and ionic membranes (EIMs) that enables the artificial recycling of CO<sub>2</sub> utilization and alleviation of metal cation stress in microalgae cultivation. HCO<sub>3</sub><sup>-</sup> was selected to transfer from the cathode chamber to the culture pond with sodium bicarbonate as the catholyte, while Na<sup>+</sup> cations were blocked with the anionic membrane in EIMs, accompanied by a gradually decreasing pH value, which facilitates microalgae growth. The reliability and universality of EIMs was further verified with both a cation-tolerant marine strain, *Dunaliella salina* HTBS, and cation-sensitive freshwater strains, *Chlamydomonas* and *Chlorella*. In particular, the cell densities of cation-sensitive strains in EIMs were much higher than those in the NaHCO<sub>3</sub> group in both 800 mL- and 150 L-scale applications, demonstrating their great potential. Moreover, the intracellular metabolites were not affected when microalgae were cultured in EIMs, implying their feasibility for commercial cultivation. Therefore, we established robust EIMs that facilitate both the efficient utilization of CO<sub>2</sub> and commercial application, which will shed light on the development of green technology for microalgae-based carbon sequestration in the future.

**Keywords: Carbon Sequestration and Utilization; Electrolysis; Ionic Membranes; Microalgae**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antimicrobial Resistance Prediction using Siamese Neural network

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### ABSTRACT

*Acinetobacter baumannii* is a type of gram-negative bacillus frequently found in hospital settings and among hospitalized patients. This bacterium has the ability to infiltrate open wounds. Its shape resembles a combination of a rod and a ball. It has rapidly developed resistance to numerous antibiotics.

Antimicrobial resistance (AMR) stands as a significant global challenge that poses a threat to human and animal health. The urgent necessity for rapid and precise AMR diagnostic techniques is evident. Traditional methods, such as antimicrobial susceptibility testing (AST), are time-consuming, have limited throughput, and can only be applied to cultivable bacteria.

However, machine learning (ML) approaches present promising opportunities to tackle this issue by enabling automated prediction of AMR using bacterial genomic data. In most ML approaches, the input consists of the bacterial genome and information regarding the antibiotic under consideration. The ML models then predict whether the bacteria are resistant to the specific antibiotic.

Still a significant challenge in this field is the limited availability of bacterial data, which results in difficulties in training robust and accurate ML models. Addressing this data scarcity is crucial to enhance the performance and reliability of AMR prediction models.

To address the challenge of limited data, this study introduces a novel approach utilizing a Siamese neural network. The network is designed to take two bacterial genomes as inputs, aiming to predict whether these bacteria demonstrate resistance to a specific antibiotic. The Siamese architecture enables a comparative analysis between the genomes, allowing the model to capture nuanced variations and patterns that potentially contribute to antimicrobial resistance.

By leveraging this innovative network design, our goal is to significantly improve the accuracy and efficiency of predicting bacterial resistance profiles. Ultimately, this approach can aid in the identification of effective antibiotic treatments, thereby mitigating antimicrobial resistance.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Applications of heterotrophic microorganisms to the mineral processing

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### ABSTRACT

Biotechnological processing of minerals is generally considered an environmentally benign approach to concentrate valuable minerals, extract valuable metals from ores and secondary resources, or to remove contaminants from industrial minerals. The method may have lower energy consumption and operational costs in comparison with the conventional chemical processes. Despite the varied application of the chemolithoautotrophic microorganisms in the acidic dissolution of base metals mostly from sulfidic resources, or bio-recovery of metal bearing minerals, chemoorganoheterotrophic microbes can be applied to a wider range of sources including ores, wastes, and industrial residues to beneficiate valuable minerals or to dissolve various elements. Nevertheless, the requirement of neutral conditions and sugar-containing media enhances the risk of microbial contamination and limits the development of heterotrophic bioleaching on an industrial scale.

### BACKGROUND AND OBJECTIVES

Heterotrophic microorganisms such as fungi and bacteria exploit organic carbon as an energy and carbon source. They degrade the organic substrates to produce metabolites which can interact with minerals. Microbe-mineral or metabolite-mineral interactions are applied to different mineral beneficiation and hydrometallurgical processes such as bio-flocculation, bio-flotation, bio-sorption, bioleaching, and bio-precipitation.

Heterotrophs may leach elements from resources through the bio-electrochemical reduction of metal oxide compounds. Reduction of ferric to ferrous iron by some bacteria in anaerobic condition or by oxalic acid secreted to the culture medium is an example. Protonation is another way to solubilize metals from minerals. Acidic metabolites generate  $H^+$  that attacks the mineral surface and destabilize the atomic bonds of the mineral crystals. In contrast to acidification, microbes can alkalify their growth environment by degrading proteins to ammonium ions using the protease enzyme. The alkaline conditions may lead to the dissolution of quartz or plagioclase minerals. The other leaching mechanism is metal complexation by organic ligands or chelators excreted to the culture media. They form stable complexes which enhance metal dissolution.

Furthermore, the microbial cells per se and their extracellular polymeric substances can be applied as flotation or flocculation reagents. Proteinic and polysaccharidic substances may be absorbed on the mineral particle surface and make them hydrophobic or hydrophile, respectively. Being adsorbed on the mineral surface, the gram-negative and positive bacterial cells may act as depressants or collectors depending on the cell wall composition.

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Despite the undeniable capabilities of the heterotrophic microorganisms in metal leaching, there are some constrictions to apply them on industrial scales. The main reason is that the conditions they need to survive, and flourish are also suitable for many other organisms. Therefore, sterilization is required to prevent microbial contamination. However, sterilization of the ores and culture media is nearly impossible.

## MATERIALS AND METHODS

The present paper reviews some recent achievements of the author and his team in the application of heterotrophic microorganisms to the biological leaching of metals from ores and secondary resources.

## RESULTS AND DISCUSSION

*Aspergillus niger* is one of the widely used fungi which is applied by the researchers around the world due to its high organic acid production capabilities that can be applied to leach various metals from primary and secondary resources.

Hosseini et al. (2007) employed *A. niger* isolated from pistachio shell and NCIM548 to remove iron contaminant from a kaolin sample. About 43% of iron was removed after one month using the strain isolated from pistachio shell at 20 g/l pulp density. Results are illustrated in Fig. 1. The continuous decline of pH during the process was the result of oxalic and citric acid excretion to the medium. The mentioned organic acids can dissolve iron oxides/hydroxide from the ore by complexation, reduction, or protonation mechanisms.

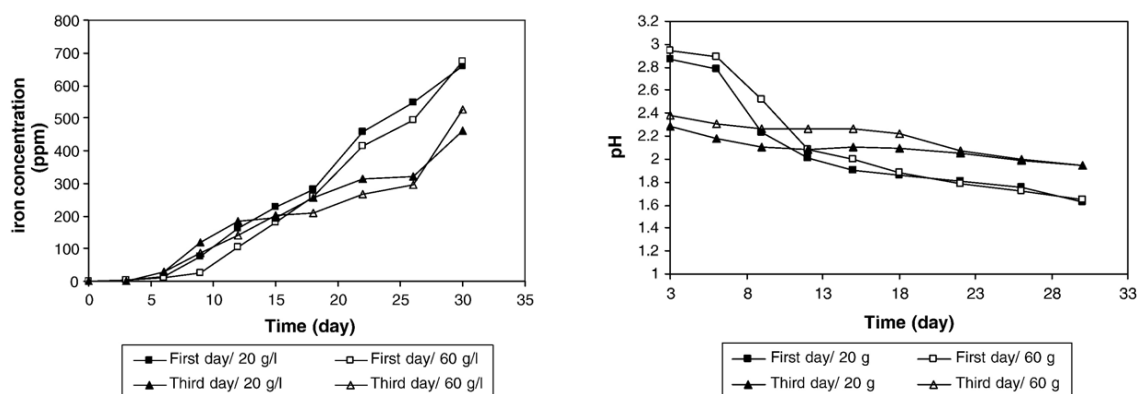


Fig. 1. Variations of (left) iron concentration and (right) pH in the culture medium of *A. niger* containing kaolin sample

Later in 2009, Aghaei et al. applied response surface methodology to model and optimize the oxalic and citric acid production by *A. niger* (isolated from pistachio shell) along with iron removal.

Fig. 2 shows the application of the *Bacillus licheniformis* cells and metabolite to hematite and goethite flocculation investigated by Sadeghizadeh et al. (2017). Obviously, increasing pH

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value enhances the settling of both minerals by different bioreagents. Also, the bacterial cells and polysaccharides have the highest flocculation effect on either hematite or goethite.

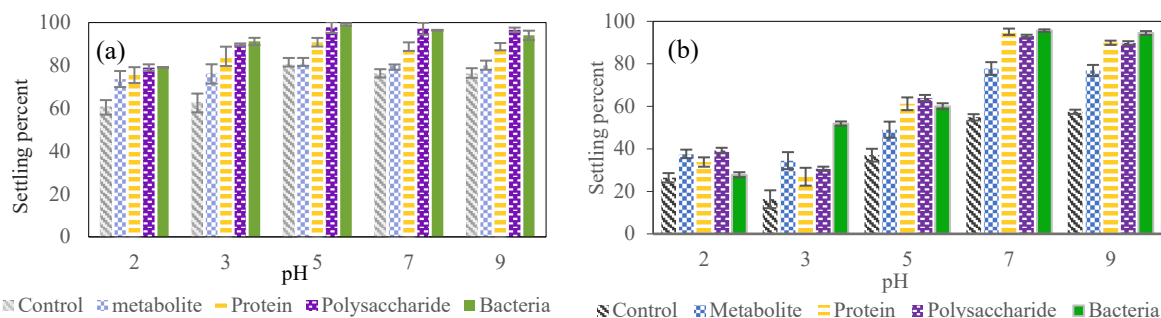


Fig. 2. Settling of (a) hematite and (b) goethite as a function of pH and type of bio-reagent

Akbari et al. (2007) investigated the biological sedimentation of kaolin and quartz using the same reagents. As can be discerned from Fig. 3, the highest settling percentage of both minerals happened by using bacterial cells. Proteins showed the lowest flocculation ability amongst the examined bioreagents. Also, the best kaolin and quartz flocculation efficiencies were observed at pH=5, and 2, respectively.

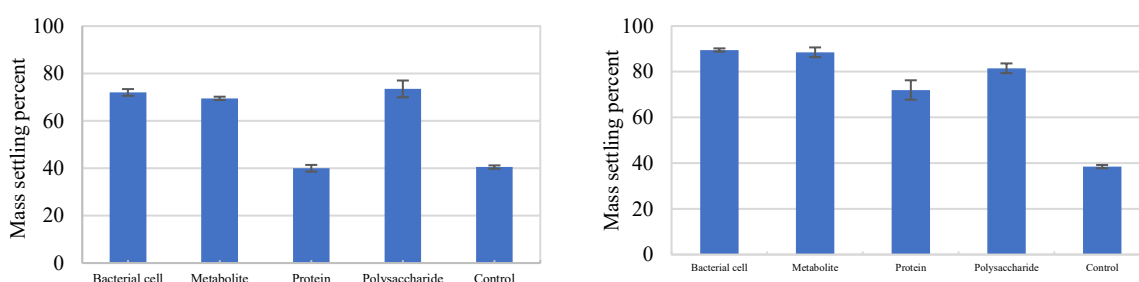


Fig. 3. Settling of (a) kaolin and (b) quartz as a function of bio-reagent at pH=5, and 2, respectively

The outcomes of two previous research works were implemented by Hosseini et al. (2019) to biologically separate hematite or goethite from kaolin and quartz. Fig. 4a, and b indicate that when bacterial polysaccharide is used as the reagent, the artificial binary mixture of the minerals is effectively separated at pH=7.

Additionally, Fig. 5 indicates that the bacterial cells and polysaccharides of *B. licheniformis* can make a significant difference between the kaolin and quartz sedimentation. This difference helps the separation of two minerals from each other.

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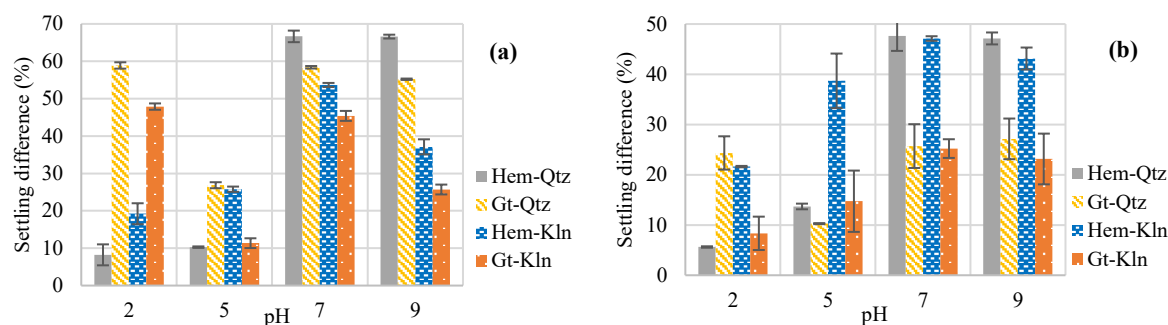


Fig. 4. Histograms of the settling differences of the iron oxides and kaolin/quartz in the presence of the bacterial polysaccharide with a concentration (a) like the metabolite and (b) like the bacterial protein

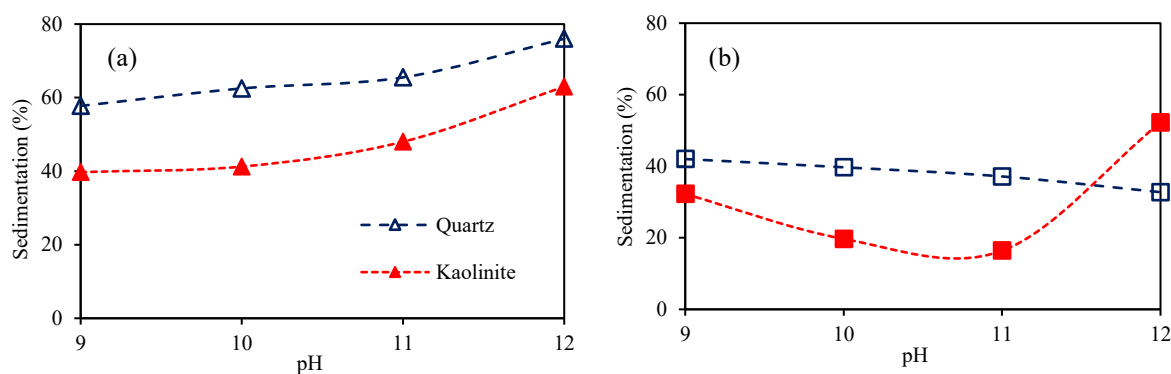


Fig. 5. Kaolinite and quartz sedimentation in the presence of different biofloculants as a function of pH (a) bacterial cell, and (b) EPS

Gorji et al. (2020) investigated the ability of *Bacillus megaterium* and *Pseudomonas aeruginosa* as two cyanogenic bacteria to leach gold from pure gold and a copper-gold ore sample in the presence of residual glycine.

Results are presented in Fig. 6. In comparison with the control tests which are indicated by square signs, both heterotrophic bacteria leached gold and copper from the ore sample. Considering gold bio-cyanidation using 1% solid content, the efficiency of *B. megaterium* was higher than the other strain.

After 108 h, the Au recovery attained more than 40% and Cu recovery reached near 60% using the metabolite of *B. megaterium* produced in 24 h. It is speculated that the synergistic effects of biological cyanide generated from glycine and residual glycine remained in the solution improved the gold dissolution efficiency.

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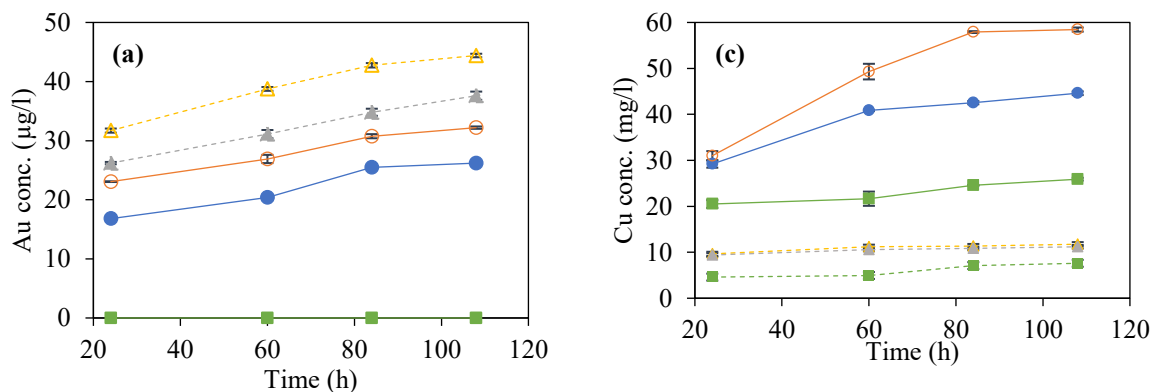


Fig. 6. Bio-cyanidation of copper-gold ore by *B. megaterium*. (a) Au concentration, and (b) Cu concentration

The ability of *A. niger* to generate organic acids such as oxalic and citric acid was applied to the leaching of valuable elements from red mud by Pedram et al. (2020). According to Fig. 7, the fungus was able to leach about 90% of Al and V, ~70% of titanium, and 65% of strontium from bauxite residue.

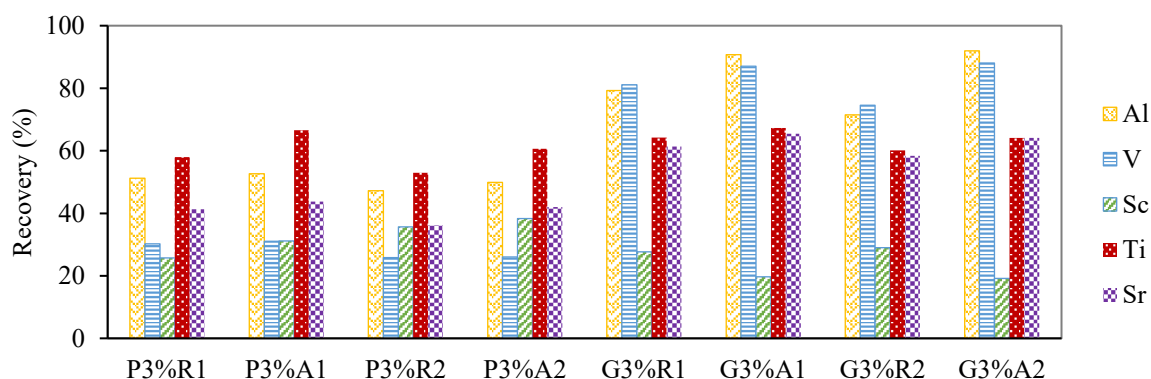


Fig. 7. Final metal recoveries of the bioleaching tests using *A. niger* isolated from the (a) Pistachio husk and (b) grape skin after 20 days.

In another interesting work, Allameh et al. (2020) implemented *B. megaterium* to synthesize ammonia and cyanide in a vinasse medium and then apply them to the ammoniacal cyanidation of a copper-gold ore sample. As a result, 97% gold recovery was achieved. The changes in cyanide and ammonia concentration during the bacterial growth are presented in Fig. 8. As seen, 30 mg/l cyanide was produced after 24 h. Also, the ammonia concentration continuously increased till the end of the experiment.

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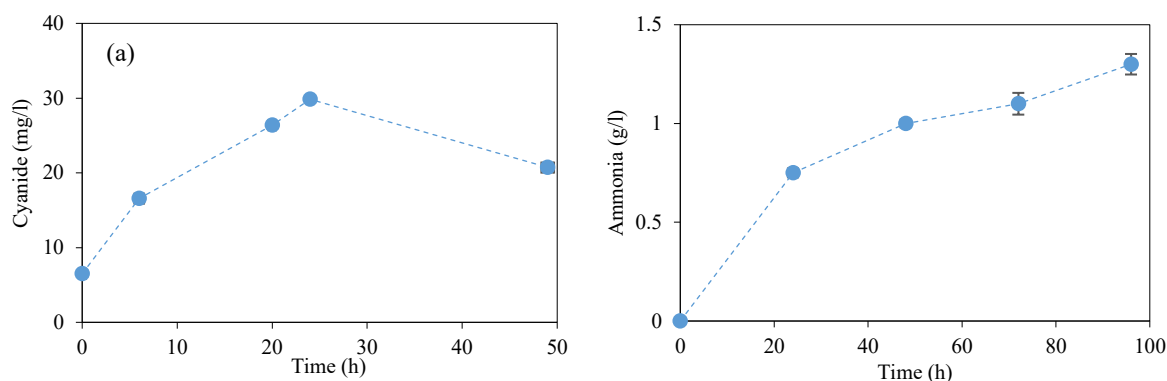


Fig. 8 Biological production of cyanide and ammonia by *B. megaterium*

Furthermore, Abedi et al. (2022) investigated the separation of quartz and barite using bio-flotation approach. They used *B. licheniformis* cells and metabolite as the bio-collector. As illustrated in Fig. 9, the bacterial cells are able to effectively separate two minerals at pH=3.

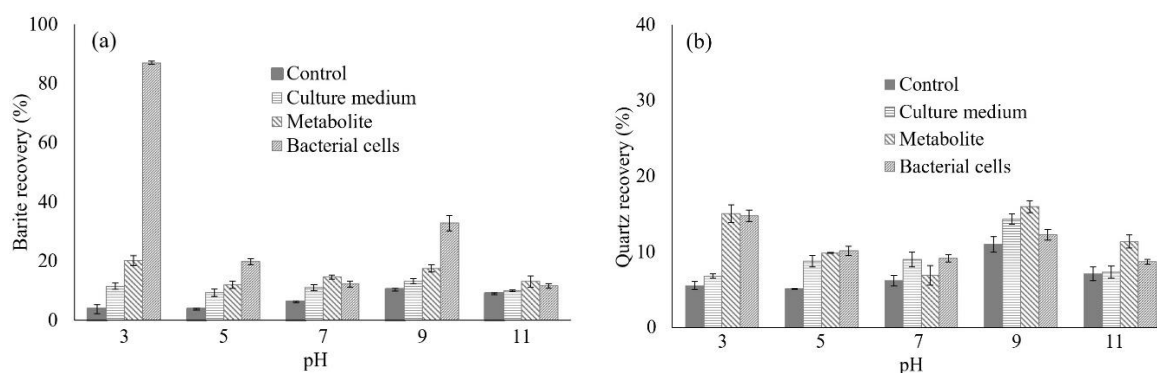


Fig. 9. Flotation recoveries of minerals as a function of pH and bioreagent type, (a) barite, (b) quartz

## CONCLUSION

The heterotrophic microorganisms can be applied to different mineral dressing and hydrometallurgical processes due to their ability to generate metabolic substances such as polysaccharides, proteins, acids, etc. As described in the present paper, the bacterial cells and metabolites can be applied to bio-flocculation and bio-flotation. Also, the organic acids secreted by fungi can be implemented to leach valuable metals from ores and secondary resources.

**Keywords: Bacteria, Bioleaching, Fungi, Heterotrophs**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Applications of Integrative Systems Biology in the analysis of Microbiome

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The human microbiome is a complex community of bacteria, archaea, viruses, and fungi that live in the body. This presentation explores the significant role of the human microbiome in the context of metabolic diseases, emphasizing the need for a multidisciplinary approach that combines diverse biological data, computational methods, and biochemical knowledge. By integrating high-throughput omics data and optimization techniques, the goal is to gain a comprehensive understanding of the microbiome's impact on human health, paving the way for advancements in personalized medicine and innovative probiotic therapies.

### MATERIALS AND METHODS

One of the key methods used in integrative systems biology is Constraint-Based Reconstruction and Analysis (COBRA). Employing COBRA in microbial community modeling allows us to harness optimization techniques to unravel complex community-level interactions, including metabolite exchange and cross-feeding among species. The process begins with the construction of genome-scale metabolic models (GEMs) for individual species. However, the true strength of COBRA emerges when these GEMs are coupled with optimization methods, particularly Flux Balance Analysis (FBA), to predict the optimal distribution of metabolic fluxes for each species. This allows for the exploration of microbe-microbe, diet-microbe and microbe-host interactions within microbial communities and their host environments, providing insights into complex metabolic dependencies.

### RESULTS AND DISCUSSION

In this presentation, we will delve into some initial findings concerning the common proposed mechanisms by which the gut microbiome may instigate the onset of obesity. The results of COBRA studies shed light on the intriguing connections between the composition and function of the gut microbiome and the development of obesity. By employing optimization techniques in bacterial community modeling via COBRA, researchers can engineer community-level metabolic behaviors, impacting drug design and enhancing our understanding of the human gut microbiota.

### CONCLUSION

Obesity is a complex and multifaceted health issue, and recent research has suggested that the gut microbiota plays a pivotal role in regulating metabolism and energy balance. This presentation underscores integrative systems biology's central role in comprehending the microbiome's influence on metabolic diseases, with a specific focus on obesity. Additionally, the examination of the gut microbiome's link to obesity highlights the transformative potential of integrative systems biology in healthcare and personalized medicine.

**Keywords:** Metabolic Network, Obesity, COBRA, Bacterial Community, Gut Microbiota

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### **Bacteriophage – Delivering Hydrogel offers simultaneous promise against methicillin-resistant *Staphylococcus aureus* (MRSA) causing bedsore and diabetic wounds**

**Dr. Morvarid Shafiei**

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#### **ABSTRACT**

#### **BACKGROUND AND OBJECTIVES**

*Methicillin* resistant *S. aureus* (MRSA) has become endemic today in hospitals worldwide. Currently, any particular antibiotic cannot be recommended for treating MRSA. Phage therapy has gained interest as an alternative treatment for MRSA infections. Hydrogels resemble living tissues by holding a high proportion of water content within its matrix, providing optimal environment for accommodating proteins, living cells and other biomolecules. In this study the antibiofilm effect of Bacteriophage – delivering Hydrogel in vitro and in vivo using an experimental mouse wound infection model was investigated.

#### **MATERIALS AND METHODS**

Bacteriophage was isolated from hospital sewage. Lytic activity and the titers of phage lysates were measured using spot test and double-layer plaque assay. The phage characterization was determined through transmission electron microscopy. Adsorption rate, host range and stability tests were investigated. The latent period and burst size were estimated from a one-step growth curve. The effect of bacteriophage against MRSA biofilms was determined.

The chitosan based hydrogel containing bacteriophage has been developed as bioresorbable local bacteriophage delivery system.

#### **RESULTS AND DISCUSSION**

TEM results showed that the phage resembled the *Cystoviridae* family. Its latent period was 30 min, corresponding to about 71/43 phage particles per infected cell. The phage had a broad host range and it was most stable at 37°C and pH 7. It was sensitive to NaCl concentrations. Release of phage from hydrogel was gradual and consistent over 21 days. The hydrogel achieved a 5.3-fold reduction in live MRSA counts at the infection site compared to bacteriophage-free hydrogel.

#### **CONCLUSION**

These results support the development of bacteriophage-delivering hydrogels to treat MRSA biofilm associated infections.

**Keywords: bacteriophage, biofilm, hydrogel, MRSA**

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### **Biobanks for “Bio-vention”: Culture Collections, professionals underpinning microbial resources exploitation.**

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#### **ABSTRACT**

Faced with global challenges such as the overexploitation of resources and climate change, countries and international institutions are reacting by developing strategies aimed at correcting and structuring societal models that are more respectful of the global ecosystem.

Several models are emerging in particular: Knowledge Based Bioeconomy (KBBE) and Bio-Circular-Green Economy (BCG). KBBE<sup>i</sup> can be concisely defined as “transforming life sciences knowledge into new, sustainable, eco-efficient and competitive products”. BCG is an Integration of bioeconomy, circular and green economy<sup>ii</sup>.

The collections adapt to the evolution of the life sciences and the changes this implies in terms of scientific needs and take account of socio-economic legal and political constraints to best meet the demands of their users. As a result, Culture Collections have evolved from mere centres of conservation and distribution of microbiological material to “Biological Resources Centres (BRC)”, basic infrastructures for biosciences in Knowledge Base Bio-Economy conceived as sources of all essentials for Research and Development in Life Sciences<sup>iii</sup>. Since 2018, the ISO 20387:2018 standard entitled "General requirements for biobanking" has been developing the biobank concept, giving a socio-economic stakeholder dimension to collections. This approach not only adds the role of wealth producers to that of knowledge producers, but also facilitates private-public partnerships. The shift of terminology reflects this evolution.

Comprehensive exploration and structured study of microbial diversity implies access to huge numbers of specimens. These assets of fundamental scientific importance must be conserved and provided with the highest level of reliability to ensure consistent Research and Innovation.

When users search through biobank catalogues, it is like looking through open windows and discovering what the microbial world has to offer. Yet scientists are rarely aware of the enormous work behind the list of strains they consult. The biobank catalogues are the sum of many efforts.

The information system stores and processes not only scientific and technical information, but also administrative and clerical data to ensure that the material provided is fit-for-purpose from both a scientific and legal perspective. The material is well identified and named according to the updated nomenclature, has the characteristics expected both for trials and for standardized industrial production under safe conditions and respecting the intellectual property rights of each.

When entrepreneurs and technicians eventually receive the microorganisms, they receive the keys to exploring and exploiting the microbial world through the professional dedication of the door openers that are culture collections.

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The ability to provide this triangle of biological material, related data and expertise transforms the passive role of collections into a full partner in the process of bio-based invention, of bio-invention. Furthermore, when this inventive process is anticipated or generated by collections, either on their own initiative or in public-private partnerships, in a Bio-Circular-Green economy, we can call it bio-venture.

- <sup>i</sup> In KBBE, “Knowledge based” refers to the increasing amount of data on biological material produced as research outputs, and processed by analytical tools, which themselves generate even more data and metadata to be managed and analysed by powerful computational tools. The term “bioeconomy” includes all industries and economic sectors that produce, manage and exploit biological resources (agriculture, food, pharmaceutical, cosmetic, and other bio-based industries). Advanced biotechnology is breaking new ground in understanding microbial diversity and bioprocesses that could lead to valuable bio-products and biomaterials. Applying such new knowledge to the production and conversion of bio-resources can boost bioeconomy and create new industries.
- <sup>ii</sup> Bioeconomy involves the production of renewable biological resources and the conversion of these resources into value added products. Circular economy aims at reusing and recycling materials to maximize the value of limited resources. Green Economy determines to keep economy, society and the environment in balance leading to sustainable development.
- <sup>iii</sup> Biological Resource Centres Underpinning the future of Life Sciences and Biotechnology, 2001, OECD, Paris. <http://www.oecd.org/dataoecd/26/19/31685725.pdf>  
OECD Best Practice Guidelines for BRC, 2007, OECD, Paris. <http://www.oecd.org/dataoecd/6/27/38778261.pdf>

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Bioremediation In Nuclear Industry by Seaweeds Biomass and Cyanobacteria

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## ABSTRACT

Biological and Biotechnological processes such as biosorption and phytoremediation as bioremediation techniques along with physical and chemical methods in the nuclear waste management process have been promising in recent years. A lot of research has been done on the use of different types of seaweeds, cyanobacteria and other microorganisms for the adsorption of radionuclides and heavy and toxic metals due to their high adsorption capacity and economy. Among the studied bioremediation methods, brown algae biomass is one of the main alternatives for the adsorption of radionuclides and heavy metals in the management of nuclear waste and other industries.

## BACKGROUND AND OBJECTIVES

The effluent released from the nuclear industry, uranium mining, power plant effluents, medical research laboratories and nuclear incidents is more harmful to the environment as well as to humans directly due to emissions. In order to deal with the harmful effects of radioactive waste, waste management is done with special physical, chemical, and biological methods[1-5].

Bioremediation and phytoremediation are the two main techniques of using microorganisms and plants to remove radioactive and heavy metals pollution. The use of dried biomass of seaweeds has been interesting due to its abundance, economic aspects and effectiveness in the treatment of radioactive liquid waste. Based on the blooming of cyanobacteria phenomenon due to the production of a high volume of biomass and their resistance to environmental unfavorable conditions is a suitable alternative for adsorbing radioactive pollution[6-12]. The objective of this lecture is to introduce some advances in nuclear wastes management to radionuclide removal by seaweeds and cyanobacteria as a biological method.

## MATERIALS AND METHODS

In order to investigate the performance of algae, cyanobacteria or microorganisms in the adsorption or bioaccumulation and removal of radionuclides, brown algae, and cyanobacteria biomass is placed in contact with the pollutant. Effective parameters such as pH, pollutant concentration, amount of adsorbent and contact time are studied to determine the optimal conditions of adsorption. Biosorption is investigated during two processes, including investigation in a batch system and an adsorbent column.

## RESULTS AND DISCUSSION

The obtained results show the high efficiency of brown algae biomass in adsorbing and removing radionuclides. Cyanobacteria have also shown an expected performance in adsorbing radionuclides. The research results showed the efficiency of algae in adsorbing uranium from 150 to 318 mg/L and other radionuclides depending on the type of biomass and operational conditions[9, 13-18]. Cyanobacteria provide the possibility of adsorption of radionuclides in the aquatic environment in the form of biological adsorption and bioaccumulation[6, 7, 12]. Reducing the volume of solid cellulose waste is also one of the cases that provides the possibility of using it with the help of microorganisms to biodegrade organic materials[3, 19-24].

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### CONCLUSION

Biotechnological processes or biosorption are proposed as effective and in many cases cost-effective options for the treatment of radioactive wastes and the removal of heavy metals. In the nuclear waste management process, brown algae have shown better performance in the adsorbent columns. It is possible to use algae and other microorganisms and plants in the bioremediation process in order to remove nuclear pollution and treat solid and liquid waste.

**Keywords: Biosorption, Adsorption, Radionuclides, Bioremediation, Removal**

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### Copper bioleaching, a sustainable way to develop copper industries

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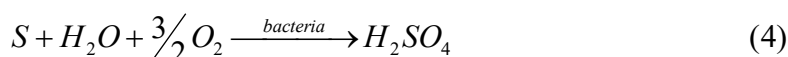
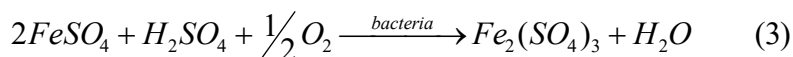
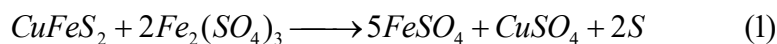
#### ABSTRACT

Increasing in copper price in international markets causes a great interest in the production of this metal as an important material with a vast application. In mining industry, chalcopyrite (CuFeS<sub>2</sub>) is the most abundant and refractory copper sulphide mineral that is the source of more than 70% of produced copper in the world.

Presently, pyro metallurgical methods are preferred to produce copper from sulphide concentrates containing chalcopyrite as the main copper sulphide. Hydrometallurgical alternatives are under consideration by copper producer. Hydrometallurgical methods are safer than pyro metallurgical methods regarding the environmental concerns. In recent decades, bacterial leaching of sulphide minerals has been extensively taken into consideration, as it is a process with low capital and operating costs and environmental benefits. This technology is not applied for chalcopyrite, because of its natural refractory against the chemical and biological leaching that decrease the rate of dissolution.

Bioleaching consists of the application of sulfur and iron oxidizing microorganisms for accelerating the dissolution of valuable metal species, like copper, zinc, cobalt or nickel from sulfide ores and flotation concentrates. Oxidation of metal bearing ores is accomplished by chemical reaction with ferric ions and/or protons (equations 1 and 2).

Microorganisms have catalytic effect and reproduce oxidation agent. It means they oxidize ferrous into ferric ions (equations 3); they also have the ability of oxidizing elemental sulfur to sulfate and producing acid (equations 4).



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Bacterial leaching of metal sulfides is used in commercial scale and has a lot of economic benefits in comparison with other technologies such as high pressure oxidation. Bioleaching of metal bearing sulfides includes agitated tank, heap and dump leaching. Selection of the leaching process is affected by grain size and ore grade. Historically, chalcopyrite has proved a stubborn candidate for obtaining high recoveries using normal bioleaching practices, either in ore heap leaching or using agitated reactors for concentrate treatment.

Metal extraction from high grade ores causes the decrease in these resources. So, the metal extraction from low grade ores has been necessary. Large amount of low grade ores is produced every year while the usage of common processing methods is not economically viable because of the low concentration; therefore, other techniques must be employed. Bioleaching is an economical and a simple technique which attracts many attentions in mining industries. Leaching in heaps offers some advantages like simple equipment, minor investment and operational costs and reasonable process recovery; hence they are suitable for low grade ore treatment. Bioleaching of low grade chalcopyrite ore (0.24% Cu as chalcopyrite, and 2.19% Fe as pyrite) have been investigated using moderate thermophilic bacteria in the column reactors. The results showed that 69.68% of copper were extracted during 6 months by the use of particles finer than 12.07mm. To develop this technology to industrial scale, a 1000 tone pilot heap was constructed. The ore contained 0.298% Cu as chalcopyrite, and 4.6% Fe as pyrite. Over than 50% copper recovery was achieved in 120 days of optimum operation.

Over the past two decades, the optimization of bioleaching processes for the treatment of chalcopyrite ores and concentrates has been the subject of many research programs. Using a flotation concentrate containing 46% chalcopyrite 23% pyrite, the high pulp density (15%) bioleaching tests were carried out at 47°C using a set up consisted of 3 continuous stirred tank bioreactors in series. To increase the copper recovery in contrast to the conventional bioleaching (~39.62%), the effect of redox potential on the chalcopyrite bioleaching was investigated by electrochemically controlled bioleaching. The results showed that by controlling the redox potential, faster copper leach kinetics could be achieved. At last, reducing the redox potential from high levels to optimum window (420-440mV SCE) caused an increase in copper recovery from around 39% to higher than 69% (over 25 g/L Cu<sup>2+</sup>)

**Keywords: bioleaching; Heap and Tank leaching; Chalcopyrite; Moderate thermophiles**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Effect of Neanderthal's Genes in Contemporary Humans Diseases

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#### ABSTRACT

This study aimed to investigate the effect of Neanderthal's genes in contemporary humans' diseases. To this end, a comprehensive literature review was conducted and relevant studies were identified from PubMed, Science Direct and Google Scholar databases. The results showed that Neanderthals have contributed significantly to the genetic makeup of modern humans and some of their genes are associated with certain diseases such as lupus, Crohn's disease, biliary cirrhosis and type 2 diabetes. Furthermore, it has been suggested that these inherited alleles could be responsible for an increased risk of developing some complex diseases in today's population. In conclusion, our findings indicate that Neanderthal's genes may play a role in influencing contemporary human health through increasing susceptibility to certain diseases. Further research is needed to better understand the relationship between Neanderthal genetics and modern human health outcomes.

**Keywords:** Neanderthal, Modern Human, *Homo sapiens*, Genes, Diseases

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## Evaluation of the effects of native probiotic bacteria on intestinal inflammatory status via different signaling pathways.

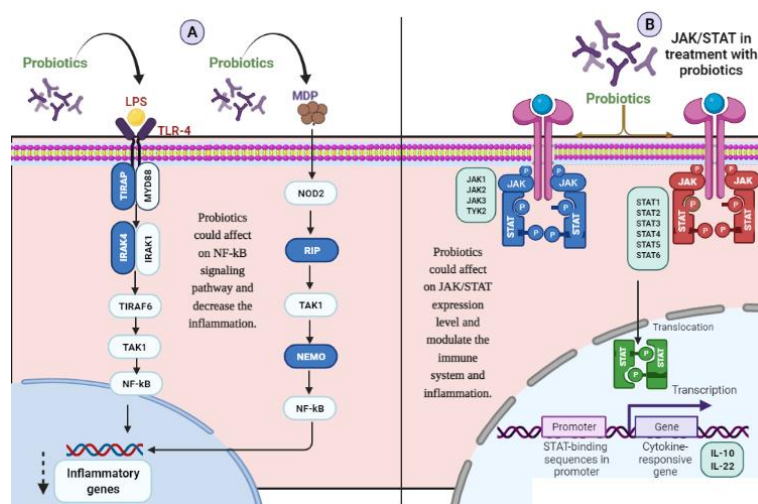
Mahdi Rohani

Associate Professor, Bacteriology Department of Pasteur Institute of Iran.

### ABSTRACT

The gastrointestinal tract (GT) is an ecosystem that contains important bacteria, including beneficial bacterial genera that have various effects on the immune system, host metabolism, and improving microbial balance. According to the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), probiotics are beneficial microorganisms and have noticeable benefits and limited side effects when used in appropriate amounts and composition. Balancing and modulating the immune system and strain-specific anti-inflammatory capabilities are two of the defining characteristics of probiotics. Our previous studies have shown the beneficial effects of probiotics in modulating and reducing inflammation in terms of phenotypic effects. However, a comprehensive investigation of the various molecular signaling pathways that play an important role in inflammation is still pending, and the effect of probiotic strains in each of these pathways should be carefully analyzed. In other words, identifying such pathways in more detail could evaluate the presumed effects of probiotics. Since the greater efficacy could be achieved when different probiotic strains are involved in a mixture, we used a mixture of *Lactobacillus* Spp. and *Bifidobacterium* Spp. Using two different species of probiotics, specifically in cocktail form, and also examining their effectiveness before inflammation have been occurred, could be useful to understand how probiotics play role as preventive agent in challengeable diseases, including IBD.

The molecular effects of our selected probiotic strains on different signaling pathways could shed light on how specific strains of probiotics can be able to preserve intestinal homeostasis by affecting pathways that is directly in communication with inflammation. We added our selected strains before, simultaneously and after inflammation to observe the native potential probiotic effects in gene expression. The results may suggest that these probiotic strains could prevent inflammation and decrease inflammation severity in an in-vitro model of inflammation. More comprehensive studies especially in-vivo models, could show the potency of our special probiotic strains consumption in preventing inflammatory-related diseases.



The overall result of probiotic treatments

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# Fungal Contributions to Sustainable Agriculture and the Challenges in Biodiversity Conservation

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### ABSTRACT

Food security remains a pressing global concern, exacerbated by the challenges posed by an expanding world population and the impacts of climate change. This paper emphasizes the pivotal yet often overlooked role of fungi in diverse ecosystems, particularly in the context of agriculture. Fungi serve as crucial agents in sustainable agricultural practices, functioning as biofertilizers, biopesticides, and inducers of abiotic stress tolerance. Arbuscular mycorrhiza (AM) fungi, dark septate endophytes (DSE), endophytic fungi (EF), and plant growth-promoting fungi (PGPF) stand out as essential biofertilizers. Beyond their growth-promoting abilities, these fungi also confer resistance against pathogens and pests, as well as tolerance to various environmental stressors. In tandem with these agricultural benefits, biocontrol fungi present a promising eco-friendly approach for managing pests and diseases. Various fungal species have demonstrated effectiveness in suppressing insects, nematodes, and other harmful organisms. Moreover, bioherbicides derived from fungal species offer a natural solution for controlling weeds detrimental to agricultural crop production.

The assessment of global fungal biodiversity and its conservation remains a longstanding challenge in the field of biology. While estimates range from 1.5 to 13.2 million fungal species, only a small fraction (1.2–10.3%) have been formally described to date. Historically, fungi received inadequate attention in conservation efforts due to factors such as insufficient awareness of their diversity, limited demographic and ecological data, and challenges associated with sampling and species identification. However, the emergence of Conservation Mycology as a dedicated subfield, coupled with advancements in molecular genetics and high-throughput sequencing technologies, has revolutionized our understanding of fungal diversity, function, and biogeography. Nevertheless, two significant hurdles persist: the potential detachment of mycologists from community analysis in the era of high-throughput sequencing, and the taxonomic ambiguity of many sequences generated. Addressing these challenges requires the standardization of the International Code of Nomenclature for algae, fungi, and plants (ICN) and a refinement of the criteria for acceptable type-specimens.

Linking genetic diversity to functional diversity represents a paramount challenge in contemporary mycological research. Genome sequencing has illuminated fungal function by predicting gene function and revealing the phylogenetic history of vital proteins, domains, and gene families. Ecosystem-level approaches offer a promising avenue for fungal conservation, capitalizing on their extensive interconnectivity with other biota through food webs and symbiotic relationships. While species and ecosystem conservation remain foundational to biodiversity preservation, safeguarding biological collections, ecological metadata, and genetic/genomic data assumes increasing importance. Herbaria, Fungaria, and culture collections are instrumental in cataloguing fungal diversity, generating knowledge, and mapping fungal distribution over time. Progress in public awareness and policy development pertaining to fungal biodiversity and ecological significance must persist, and conservation mycologists will play a leading role in integrating fungal management into existing conservation practices. This comprehensive overview underscores the vital contributions of fungi to sustainable agriculture and conservation, and calls for concerted efforts in research, policy, and practice to harness their full potential.

**Keywords:** Bioinoculants, Crop productivity, Ecosystem function, Integrative taxonomy, Plant protection, Soil health, Species diversity

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# Induction of mutations using gamma irradiation to improve the enzymatic metabolites production in microorganisms

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## ABSTRACT

### BACKGROUND AND OBJECTIVES:

Enzymes are biological polymers that usually were used pure or complex, in small amounts. Microorganisms such as *Penicillium* sp., *Trichoderma* sp., *Aspergillus* sp., *Streptomyces* sp. *Bacillus* sp. were the biological agents used for the synthesis of enzymatic metabolites (cellulase, xylanase, chitinase and pectinase, laccase, phytase and protease) which are required in agricultural industries. Natural mutation is the most important factor in the genetic changes and to create new microbial strains, but it happens at a very slow rate due to mechanisms of genome protection and genetic stability of species.

### MATERIALS AND METHODS:

To access new microbial strains with the potential to produce diverse or more efficient metabolites, induced mutation by gamma irradiation is used to produce new mutant strains.

### RESULTS AND DISCUSSION:

Induced mutation led to reduce the cost and time of screening, in studies of the secondary metabolism pathways of microbes or to produce new and more efficient metabolites needed in industries and sometimes to change the ratio of metabolites produced by microorganisms in industrial fermentation. Random and hereditary genetic mutation induction using gamma ray irradiation, screening mutants in the lab scales and optimizing the fermentation conditions of selected mutants to produce required metabolites are the common steps of obtaining strains with more enzyme production efficiency.

### CONCLUSION:

Nuclear Science and Technology Research Institute (NSTRI) has made several achievements in the field of applying the induced mutation technique with gamma irradiation and producing more efficient microbial mutants (*Trichoderma*, *Aspergillus* and *Bacillus*) for the production of various hydrolytic enzymes.

**Keywords:** Enzymes, mutation, gamma ray irradiation.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Low-grade chalcopyrite copper ore processing with hybrid bioleaching

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#### ABSTRACT

This article reviews the use of hybrid bioleaching with chloride NaCl and sulfuric media to improve the extraction of copper from low-grade chalcopyrite ores. Chalcopyrite is the most abundant copper mineral but is difficult to extract using conventional hydrometallurgical processes due to slow kinetics and low metal recovery. Bioleaching using microorganisms is suggested as a more sustainable method for chalcopyrite processing. This article summarizes findings from various studies on the effects of adding sodium chloride to chalcopyrite bioleaching systems. Key results show that NaCl can accelerate chalcopyrite dissolution by reducing elemental sulfur layers, increasing surface area and porosity, and improving overall leaching efficiency. Optimal NaCl concentrations range from 15 g/L to 120 mM depending on the study. However, high NaCl can also negatively impact microbial growth and activity. The mechanisms behind the interaction of NaCl and microorganisms during bioleaching require further research. Overall, this review highlights the potential of using hybrid NaCl-bioleaching systems to improve copper extraction from refractory low-grade chalcopyrite ores in a more sustainable manner.

#### CONCLUSION

In conclusion, this review highlights the potential of using hybrid bioleaching systems with the addition of sodium chloride for improved processing of low-grade, refractory chalcopyrite copper ores. Chalcopyrite is the most abundant copper mineral but presents challenges for extraction using conventional hydrometallurgical methods. Bioleaching with acidophilic microorganisms provides a more sustainable alternative, but chalcopyrite dissolution is hindered by the formation of elemental sulfur layers on mineral surfaces. Multiple studies demonstrate that adding NaCl at optimal concentrations between 15-120 mM can effectively increase chalcopyrite dissolution rates and copper recovery by reducing passivating sulfur layers, increasing surface area and porosity, and preventing iron hydrolysis. However, high NaCl levels can also inhibit microbial growth and activity. The interaction mechanisms between NaCl and microorganisms during bioleaching are complex and require further elucidation. Overall, this review shows that hybrid NaCl-bioleaching systems have significant promise for improving the extraction efficiency and economics of processing low-grade, complex copper ores in an eco-friendly manner. More research is still needed to optimize NaCl concentrations for maximum copper yields while maintaining active microbial communities. As global demand for copper rises, hybrid bioleaching presents an innovative and sustainable solution for exploiting low-grade ores that would otherwise be uneconomical to process using conventional methods.

**Keywords:** Chalcopyrite; Bioleaching; Sodium chloride; Copper extraction; Hybrid process.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Microbiological Perspective on the Interaction of Microorganisms with Uranium

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Today, the safe removal and recovery of radionuclides and heavy metals from the contaminated sources and ores has become a valuable issue in the environmental field. Normally, there are different methods for their removing and recovery, which are divided into two Techniques: biological methods and non-biological methods. Bioremediation and biorecovery are the growing methods with an increasing trend in studies and practical uses for the recovery and removal of radionuclides and metals. They are mentioned as the green methods (an energy-efficient, eco-friendly and efficient method). Microorganisms are known to have an important effect on the environmental fate of metals and uranium through physicochemical and biological mechanisms and can affect their toxicity and solubility. Understanding the interaction of microorganisms with uranium will be useful for the efficient treatment of radioactive waste, etc. The biological methods depend on the presence of suitable microorganisms and their appropriate performance in environmental conditions. Therefore, the use of microorganisms adapted to each environment is the most important practical approach to achieve the optimizing the processes. Microorganisms that grow in extreme environments have been shown to be remarkably resistant to a wide range of extreme conditions in many cases.

### MATERIALS AND METHODS

Oxidation, bioaccumulation, biosorption, bioreduction and bioprecipitation can be mentioned among the interactions between microorganisms and uranium that have role in mobility and immobility of this radionuclide in the environment. Understanding the optimal conditions of uranium-microorganism interactions, including environmental conditions affecting the process, is needed to develop knowledge of the biological technique. Also, the process of targeted screening and isolation of new microorganisms with unique characteristics from the environments contaminated with radionuclides is useful in these studies.

### RESULTS AND DISCUSSION

Today, it has been proven that these microbial interactions have a good potential to replace the conventional methods of removing and separating metals and radionuclides from the environments. Several reports showed that indigenous microbes in the places contaminated with toxic metals can be more tolerant to toxic metals. Today, a wide range of microorganisms

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have been identified from mines and contaminated places that can remove or immobilize uranium. It has been determined that the interactions of these biological agents with uranium can lead to uranium removal and recovery from the environments. So according to the reports, it is possible to isolate the new microbial isolates from the specific environments, which are very useful for removing and recovery of uranium.

### CONCLUSION

The abundance and diversity of microorganisms in environments contaminated with uranium and radionuclides and their resistance to the pollutants and their superior removal and recovery capacities have led to the high importance of identifying metal-resistant microbial populations and determining their metal removal/recovery potential.

**Keywords: Bacteria, Biomining, Bioremediation, Radionuclides, Uranium**

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# Multi-immobilization of Carbohydrases and Proteases by Nanomagnetic Combi-CLEAs Method for Oil and Protein Hydrolysates Extraction from Oil Seeds in Aqueous Phase

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### ABSTRACT

Functionalized magnetic nanoparticles are effective enzyme carriers since they have several advantages such as easily separation under external magnetic fields as well as enhancing mass transfer and reusability of enzymes. In this article, three types of enzymes included Viscozyme L (carbohydrases), Alcalase 2.4 L (endo-peptidase), and Flavourzyme (Exo-peptidase) Simultaneously were immobilized by nanomagnetic cross-linked enzyme aggregates (CLEAs) method. Then assessment of the results obtained was performed. At first, Fe<sub>3</sub>O<sub>4</sub> nanoparticles were synthesized by chemical co-precipitation then, surface coating procedure was used for functionalization of Fe<sub>3</sub>O<sub>4</sub> by lysine amino acid. Thereafter, enzyme aggregation and cross-linking was achieved by using of enzymes with functionalized Fe<sub>3</sub>O<sub>4</sub> and glutaraldehyde in saturated ammonium sulfate, and/or solvents such as, acetone, acetonitrile, tert-butanol, isopropanol, and ethanol at 3-4° C separately. Afterthat, for size reduction of formed NM-Combi-CLEAs, high speed homogenizer and then ultrasonic waves were applied. Then, produced NM-Combi-CLEAs was hold at 3-4° C for 3-24 hours and cross-linked enzymes were separated from liquid phase by centrifuge at 15000 rpm accurately. Finally, activity assay, FE-SEM images and EDX, DLS and zeta potential analysis, FTIR, degree of hydrolysis (DH%), kinetic parameters (K<sub>m</sub>, V<sub>max</sub>, k<sub>cat</sub>, k<sub>cat</sub>/K<sub>m</sub>, t<sub>1/2</sub>, k<sub>d</sub>) and thermodynamic parameters (ΔG, ΔH, ΔS, E<sub>a</sub>(in)) of immobilized enzymes compared to native enzymes mixtures was evaluated. The NM-Combi-CLEAs kept 75-80% of its original activity after 10 cycles, which proposes strong operational stability. In conclusion, the NM-Combi-CLEAs are thermo-stable, reusable, and efficient nanobiocatalyst for enzymatic oil and protein hydrolysates extraction in aqueous phase.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### New approaches biomining and recycling of Metals by using synthetic Biology

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#### ABSTRACT

Daniel Gibson and his co-workers at the J. Craig Venter Institute (JCVI) did just synthetic genome. Building on 15 years of investigation, the JCVI researchers used a computer to design a genome and a DNA synthesizer to make short stretches of DNA. These were stitched together to create a 1.08 million base pair genome based on the chromosome of *Mycoplasma mycoides*. The genome was transplanted into *M. capricolum*, which after a few rounds of replication consisted entirely of molecules whose synthesis was directed by a chromosome that started as computer code and four bottles of deoxyribonucleotides. The new microbe is known as *M. mycoides* JCVI-Syn 1.0, or just Syn 1.0. Synthetic biology involves the application of engineering principles to molecular biology, and the adoption of the philosophy of 'design, build, test, and learn', allowing iterative construction and feedback loop analyses. The exception to this is that of the more established BioBrick system, for which there are examples of its implementation for the bioremediation of gold, cobalt and nickel, mercury, and the detection of arsenic, mercury and copper. The use of biological organisms to recovery of metals from the environment is an important step in limiting the threat to metal criticality as well as a means for the detoxification of land and wastewater. Synthetic biology offers the genetic tools to respond to this opportunity by adapting current organisms or more model- organisms to improve this recovery process. Though at the small scale currently, its adaption to the industrial level, will require both a political and social acceptance genetically modified organism. Synthetic biology will continue to spread to an even wider number of organisms and help in the replacement of technologies currently carried out by chemical methods especially in bioleaching and bioremediation researches.

**Keywords:** Synthetic biology, Bioleaching, Contaminated soil, bioremediation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Production of paraprobiotics and vaccines using radiation technology

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Due to the limitations and challenges related to the use of drugs and chemicals, antibiotics, there has been an increase in the use of alternative immunostimulant agents. These include vaccines, live microorganisms (probiotics), indigestible fibers, short-chain carbohydrates (prebiotics), and a combination of probiotics and prebiotics, known as synbiotics.

About 1000 BC, vaccination for the smallpox virus existed in China and India. Typically, a vaccine serves to activate the immune system's response to combat specific disease. The immune response is affected by various factors, including genetic factors that regulate it, the individual's nutritional status, the presence of maternal antibodies, the type and amount of antigen, the injection method and site, and the use of substances that boost the immune system.

On the other hand, the usual definition of probiotics is live microorganisms that, when consumed in adequate amounts, provide health benefits to the host. Probiotic organisms must be alive to have a positive effect on the host. Despite numerous studies on the beneficial effects of probiotics, concerns have been raised about the use of these live microorganisms. There is an increasing level of interest in the potential benefits of non-living probiotic microbes, which are also commonly referred to as paraprobiotics. These types of microorganisms have the potential to provide advantageous effects without the concerns surrounding the viability of live probiotic species in food, the colonization patterns, and the likelihood of resistance or the transmission of virulence genes from pathogenic bacteria via horizontal gene transfer.

### MATERIALS AND METHODS

In the realm of vaccine production, a variety of organisms or their derivatives are utilized, such as living or inactivated organisms, toxoids, or a combination thereof. It is important to consider the specific properties and characteristics of each agent to ensure that the resulting vaccine is effective, safe, and free from unwanted side effects.

Inactivated vaccines are made by suspending whole dead bacterial, viral, parasitic, or fungal cells. Inactivation is the most crucial stage in the production process of these kinds of vaccines and paraprobiotics. Various techniques, such as thermal, chemical, and irradiation, can inactivate microorganisms.

Irradiation is a more efficient method to inactivate microorganisms compared to chemical and thermal methods. This process does not need genetic manipulation or the inactivation of the microorganism at various stages. The use of ionizing rays, specifically gamma radiation, exerts a potent lethal impact on microorganisms due to their high energy. Hence, this approach is highly appropriate for large-scale industrial manufacturing. The utilization of gamma rays is a reliable and secure method that boasts exceptional permeability, leaving no residual effects after irradiation. Gamma irradiation has minimal

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impact on the microorganism's antigenic epitopes, preserving its properties. These epitopes are crucial in eliciting an appropriate immune response, and any damage can weaken the vaccine's effectiveness. The utilization of radioprotective agents, such as manganese ion ( $Mn^{+2}$ ) and trehalose sugar, has been found to effectively preserve the immunogenic epitopes of microorganisms during radiation inactivation processing. But, exposure of microorganisms to irradiation leads to the loss of their reproductive ability and, consequently, prevents them from causing infections. Nevertheless, their metabolic activity remains intact, and as such, they are referred to as "non-reproducible" or "metabolically active" microorganisms. The metabolic activity of these microorganisms leads to the synthesis of functional proteins, which, following radiation exposure, elicits a broader immune response in the immunized host.

### RESULTS AND DISCUSSION

The comparison of methods for rendering microorganisms inactive in vaccine/paraprobiotics production reveals that the chemical-based approach presents a significant challenge. The challenge of penetrating the membrane of microorganisms and the impact on nucleic acid are the reasons why these substances are difficult to work with. Additionally, Residues of chemical substances present in yield (vaccines/immunostimulant agents) may have an adverse impact on the immunological proteins of microorganisms. Disposing of the remaining chemicals is a demanding and costly undertaking.

Also, protein damage during the inactivation process affects vaccine specificity and safety by compromising its structural epitopes. The utilization of radiation as a method for pathogen inactivation results in less damage to the structural epitopes, as compared to chemical inactivation methods. This is especially true when radioprotectors, such as trehalose sugar or manganese, are employed.

In light of the rising demand for irradiated vaccines, it is imperative to address the challenges that have impeded their commercial production. The living organism's exhibit varying degrees of sensitivity to radiation, which can be discerned based on their genome size. The following order can be used to indicate sensitivity: 1- single-stranded RNA and DNA viruses, 2- double-stranded DNA viruses, 3- haploid bacteria and yeasts, and 4- different cells and tissues of mammals and birds, as well as diploid yeasts, which exhibit decreasing sensitivity to radiation as we ascend the order. Therefore, to produce killed or inactivated particularly viral vaccines using irradiation, a high dose of gamma rays is required. This is a big safety problem for producer industries. Therefore, some researchers are actively exploring new electronic technologies such as X-rays to produce irradiated vaccine/ immunostimulant agents.

### CONCLUSION

By improving accessibility and availability, we can save lives with vaccines worldwide. Using radiation technology, our country too shows great potential for producing immunogenic materials (vaccines) for animals and humans. With the removal of bottlenecks, we can anticipate a future where these materials can be produced on an industrial scale.

**Keywords: irradiated vaccine, paraprobiotic, chemical, antibiotics.**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Production of plant bioactive compounds in microbial systems via metabolic engineering and synthetic biology

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## ABSTRACT

Plant natural compounds especially secondary metabolites play a key role in human life due to their applications as an additive, flavor, fragrance, pigment, pesticide and medicine. However, these compounds are produced in low quantity in native plants. With the progress in genomics, transcriptomics, metabolomics and genome editing tools, elucidation of the biosynthetic pathways genes of related compounds have been accelerated to pave the way towards re-engineering these compounds in new production platforms for commercialization. Metabolic engineering/Synthetic biology is an efficient method to produce natural compounds in a sustainable way at larger scale. As most of plant natural compounds are not biosynthesized by microorganisms, synthetic biology has been used to develop the engineered microorganisms such as *Saccharomyces cerevisiae*, *Escherichia coli* for heterologous production of plant natural compounds. In the present work I will show how to elucidate the biosynthetic pathway of natural compounds by functional characterization of the biosynthetic genes, select the proper microbial platform host and use new cutting-edge tools such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), CASTs (CRISPR-associated transposons) to integrate the plant biosynthetic genes in microbial platforms. In addition, opportunities and challenges for large scale production and commercialization will be discussed.

**Keywords:** Synthetic biology, Secondary metabolites, Biosynthetic pathway, CRISPR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### **Progressive concept of postbiotic metabolites, new horizons of applied microbiology in functional foods and nutraceuticals**

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#### **ABSTRACT**

Applied microbiology and food safety research co-target new and innovative technologies to access the microbiological safety and stability of food products. By far, the biological hazards – primarily bacteria and viruses – pose the greatest risk in food microbiology. Therefore, there is always a need to go over basic concepts to better overcome food safety hazards. Beneficial microorganisms and their functional metabolites are increasingly being considered as bioprotective agents while among different natural antimicrobials, a considerable interest has been focused on those of microbial origins due to their more renewability, lower production costs, and natural origin. Considering the safety limitations of chemical preservatives, these microbial components known as postbiotics are recently considered as a progressive approach for extending food shelf-life, developing functional foods/nutraceuticals, and boosting human health. Hence, it seems precious to evaluate the effectiveness of different probiotic-derived ingredients and their substantial potential to be incorporated in food systems as a promotion acting mechanism in retarding the growth of food spoilage and pathogenic microorganisms. The primary purpose of this presentation is to present the recent findings and our research outcomes based on several studies during the recent years, carried out on extraction of postbiotics components from traditional and/or commercial probiotics including organic acids, exopolysaccharides, bacteriocins, etc., plus their direct or indirect usage in food systems. Moreover, the effectiveness and functional impact of postbiotic application in food safety in retarding the growth of food spoilage and pathogenic microorganisms is overviewed.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Radiation application in cultural heritage conservation

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Cultural heritage (CH) is the legacy of attributes of a group or society that are inherited from past generations and maintained for the benefit of future generations. It includes 'tangible' (such as ancient buildings, works of art based on paper, textiles or wood, carpets and rugs) and intangible (such as the customs of a region) works. Studying and keeping art objects and other cultural heritage artefacts available, in the best possible condition, for future generations is a significant challenge. CH in improper conservation conditions is susceptible to biological attacks by insects, fungi and bacteria, which are accompanied by holes, discoloration, exfoliation and decay in the works.

### MATERIALS AND METHODS

One of the disinfection methods in recent years is irradiation with ionizing gamma rays and electron beams. Irradiation due to direct and indirect effects causes inactivation and destruction of microorganisms. Research in this field requires a multidisciplinary approach and taking into account ethical principles in the protection of CH. A research includes investigating the radiation resistance of dominant microorganisms, determining the required dose of disinfection, investigating the effect of radiation on the functional properties of the underlying materials of CH, and simulating the dose uniformity in CH during the radiation processing at the irradiation facility. Ionizing rays are highly penetrative, applicable at room temperature and eco-friendly.

### RESULTS AND DISCUSSION

*Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Pseudomonas*, *Bacillus*, *Brevibacillus* and *Burkholderia spp.* are some of the isolated microorganisms from ancient contaminated papers, paintings and textiles. In order to determine the required radiation dose for disinfecting CH, it is necessary to investigate the radiation sensitivity of the dominant isolates on the substrate. In this case, the minimum dose required to destroy insects is 0.5 to 2, fungi 5 to 7, and bacteria 8 to 10 kGy. The effect of radiation on the physical and chemical properties of CH is investigated separately.

### CONCLUSION

Considering the age, geographical area, diversity of inorganic and organic components used in CH, dominant microbial contamination and the type of irradiation source each work has unique characteristics which provides a new research field of study for those who are interested in.

**Keywords: radiation processing, cultural heritage, disinfection**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### **Sustainable treatment of industrial waste air using two-phase partitioning bioreactors and microbial composition shifts during the long-term operations**

**Dr. Seyed Morteza Zamir**

**Associate professor in Biochemical Engineering, Faculty of Chemical Engineering,  
Tarbiat Modares University**

#### **ABSTRACT**

Biological treatment methods are considered as promising, environmentally friendly, and cost-effective technologies for the treatment of volatile organic compounds (VOCs) from the industrial waste air. The hydrophilic pollutants, e.g., alcohols, ketons, etc., are removed effectively using common bioreactor configurations containing a bulk of water for the absorption of target compound from the air and subsequent biodegradation by the associated microorganisms. However, the removal efficiencies of hydrophobic pollutants, e.g., volatile aromatic and aliphatic hydrocarbons, decreased significantly since they are not water-soluble compounds. Two-phase partitioning bioreactors (TPPBs), including a water-immiscible and non-biodegradable organic phase, have been introduced in the past decade to overcome this mass transfer challenge. On the other hand, the simultaneous presence of hydrophilic and hydrophobic compounds in the produced waste air of some industries provides complex environmental effects on the microbial community, regarding the interactions between the components and the associated groups of microorganisms involving in the biodegradation process. This lecture focuses on the design and operation of TPPBs along with the microbial composition shifts in these systems during long-term operations.

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### Technologies for Harvesting the Microalgae for Industrial Applications

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#### ABSTRACT

Microalgae are emerging as a promising source for augmenting the supply of essential products to meet global demands in an environmentally sustainable manner. Despite the potential benefits of microalgae in industry, the high energy consumption for harvesting remains a significant obstacle. This review offers a comprehensive overview of microalgae harvesting technologies and their industrial applications, with particular emphasis on the latest advances in flocculation techniques. These cutting-edge methods have been applied to biodiesel production, food and nutraceutical processing, and wastewater treatment. Large-scale harvesting is still severely impeded by the high cost despite progress has been made in laboratory studies. In the future, cost-effective microalgal harvesting will rely on efficient resource utilization, including the use of waste materials and the reuse of media and flocculants. Additionally, precise regulation of biological metabolism will be necessary to overcome algal species-related limitations through the development of extracellular polymeric substance-induced flocculation technology.

**Keywords:** Microalgae; Harvesting technologies; Industrial application; Flocculation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The Silent Threat: Impact of *Aspergilli* Spores on Biodeterioration of Cultural Heritage

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Aspergillus* species possess a remarkable ability to grow in a multitude of environmental conditions. They disseminate through the production of large numbers of asexual spores called conidia. These spores are airborne and remain dormant until encountering favorable conditions for growth, such as optimal humidity, temperature, and nutrient availability. Upon finding these conditions, germination ensues, characterized by cellular swelling and the emergence of a germ tube.

*Aspergillus* spores are ubiquitous in cultural heritage environments due to their widespread distribution and resilience. Upon germination, the resulting hyphae can secrete acids and a plethora of enzymes that contribute to material degradation. Here we studied the minimal nutrient requirements for germination of conidia of *aspergilli* to assess whether these can be used to control the biodeterioration of cultural heritage.

### MATERIALS AND METHODS

We studied the germination of conidia of five *Aspergillus* species: *A. niger*, *A. oryzae*, *A. clavatus*, *A. nidulans*, and *A. terreus*. Utilizing the oCelloScope imager and computational models, we analyzed their minimal nutrient requirements for germination, in particular using amino acids as signaling molecules and/or carbon source. We examined germination in pure water and observed the effects of various nutrients, including glucose, phosphate, nitrate, and sulfate.

### RESULTS AND DISCUSSION

Our study revealed varying germination potentials among the five *Aspergillus* species in both pure water and nutrient-supplemented conditions. For instance, *A. clavatus* and *A. nidulans* were able to germinate in pure water, while *A. niger* conidia required a combination of an inducing carbon source and either inorganic phosphate, nitrogen, or magnesium sulfate for germination. Alanine and proline were found to be the most effective in inducing germination in most species except *A. terreus*. In contrast, leucine, isoleucine, cysteine, and methionine were generally the least effective. Results showed that not only the nutrient composition affected germination incidence but also the spore concentration. In fact, both inter- and intra-species heterogeneity was observed in the latter case. Results indicate that spores of *Aspergillus*

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use a bet-hedging strategy. Only part of the spores germinate. This ensures that the substrate can be colonized by the germlings on the one hand, but that stress-resistant spores are still present that ensure survival when humidity or nutrient conditions change.

### CONCLUSION

Our findings suggest that *Aspergilli* exhibit distinct competitive potentials across different substrates, utilizing a bet-hedging strategy in their germination response. Also notable, spores of *Aspergillus* can inhibit the germination of their own and that of other species. These insights are crucial for understanding the biodeterioration risks posed by *Aspergillus*, particularly in the context of cultural heritage preservation. The fact that spores of different *aspergilli* respond differently to environmental conditions hampers a generalized control of their germination to prevent damage of cultural heritage.

**Keywords:** *Aspergillus*, Germination, Spore, Biodeterioration, Cultural Heritage

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# Thermoacidophilic bioleaching of copper sulfide concentrate in the presence of chloride ions

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### ABSTRACT

The role of chloride ion in the performance of extreme thermophiles bacterium *Sulfolobus acidocalarius* in bioleaching process of copper sulfide concentrate at Midouk Shahr-e-Babak Complex was investigated. The gradual adaptation of bacteria to chloride ions at pH=1.5 showed that the presence of chloride ions in solution reduced the reproduction and growth rate of bacteria but did not prevent their growth. Results indicated that the effect of decreasing pH from 2.0 to 1.5 on bioleaching of copper sulfide concentrate is to increase the recovery of copper in the first few days, and nearly 100% of copper was extracted after 9 d. As the solid content in solution increases from 1% to 3%, about more 6 d was required to extract copper. Bioleaching of copper sulfide concentrate revealed that the dissolution of copper sulfide concentrates at constant pH=1.5, 1% solid content, and concentration of 0.5 mol/L and 1.0 mol/L NaCl after 9 d, was 98% and 80%, respectively; and after 21 d, it reached nearly 100% and 90%, respectively. Under the same conditions without microorganisms, copper extraction reached 62%.

### BACKGROUND AND OBJECTIVES

Since common and traditional methods for extraction of heavy metals from ore are no longer economically feasible, and using pyrometallurgical methods for copper production from copper sulfide minerals leads to environmental problems and pollutant generation, using alternative methods, such as hydrometallurgy and biohydrometallurgy, is gaining interest. Biohydrometallurgy is applicable to production, extraction, and recycling of different metals from minerals and industrial wastes [1-4]. There are many reasons to consider biohydrometallurgy as a suitable method for extracting metals from low-grade ores and sulfide concentrates, such as copper grade reduction in ore, less energy consumption and less pollution to the environment. Hydrometallurgy and biohydrometallurgy need a lot of water. However, in many cases, supplying low-salinity water is difficult for industries. Usually, the water used contains large amounts of different ions including chloride. In this paper, bioleaching tests were conducted on sulfide concentrate produced at the Midouk Copper Complex in Shahr-e-Babak City located in a dry area in Southern Iran. The Midouk Copper Complex must use underground water resources which contain chloride ions. An alternative water supply being considered is

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the piped Persian Gulf seawater. A case study was done to investigate the effects of chloride concentrations and pH on dissolution of copper from the copper sulfide concentrate. The unique feature of this research is that the copper sulfide concentrate has different kinds of copper sulfide minerals, such as chalcopyrite, covellite, and bornite. Moreover, bioleaching of copper sulfide concentrate was carried out using the native extreme thermophile bacteria *Sulfolobus acidocaldarius*, which was adapted in the presence of high chloride concentrations. The bacteria were isolated from acid mine drainage of the Sarcheshmeh Copper Complex located near the Midouk Copper Complex.

### MATERIALS AND METHODS

Some tests were designed to determine the effects of different parameters on bioleaching copper sulfide concentrate from Shahr-e-Babak, Midouk Copper Complex. Two different levels of parameters such as initial pH, solid content, and sodium chloride concentration were studied. pH value of the samples aided by concentrated sulfuric acid was measured daily for 21 d using Soil pH meter PRN-41. After sampling, 5 mL of distilled water was added to keep the volume of the solution constant. Tests 12 and 13 were done without chloride ions using the same method as tests 2, 4, 5, and 6, with the exception that sampling was done only at the end of the 21st day. Samples were poured to 15 mL falcon tubes, where the solid material was separated using a centrifuge (Sigma 2– 16 PK, Germany) at 6000 r/min in 3 min. The sample solutions were also filtered using a syringe and 0.22  $\mu\text{m}$  syringe filter (Biofil, Canada) to ensure sample clarity. The concentration of copper in each sample was determined with ICP-OES analyzer. The leaching tests were done to compare the results of chemical leaching and bioleaching of the Midouk copper sulfide concentrate. In test 9, 1 g of copper concentrate (1% w/v) and 3 g of NaCl (0.5 mol/L) were added to 250 mL Erlenmeyer flask containing 100 mL of distilled water. pH of solution was set at 1.5 using concentrated sulfuric acid. Then 50 mg/L of thymol and 5 g/L of citric acid were used as antibacterial agent to prevent microorganism from growth in the solution. The Erlenmeyer flask was put inside the shaker-incubator at 60 °C and under agitation rate of 150 r/min for 21 d. Sampling and other steps were similar to previous bioleaching tests 1 – 8. Tests 10 and 11 were carried out using the same method as test 9 but without chloride, and sampling was done only at the end of the 21st day.

### RESULTS AND DISCUSSION

#### 1- Effect of solid content on bioleaching

The bioleaching tests were carried out with 1% and 3% solid content, 0.5 and 1.0 mol/L NaCl, and initial pH=1.5 and 2.0 (Figure 1). According to Figure 6, Cu extraction increased with time and reached faster its maximum value (90% to 100%) in the tests with 1% solid content compared to the tests with 3% solid content, and then remained constant with time. It may be because chalcopyrite dissolves faster in the lower solid content tests. However, the tests with 3% solid content needed more time to reach the maximum Cu extraction (about 6 d). Since increasing solid content also means increasing tension on the cell walls of the bacteria, *Sulfolobus acidocaldarius* dissolved more copper in the 1% solution compared to the 3% solution, in a shorter period of time. It is also noted that during these tests, a reduced mass

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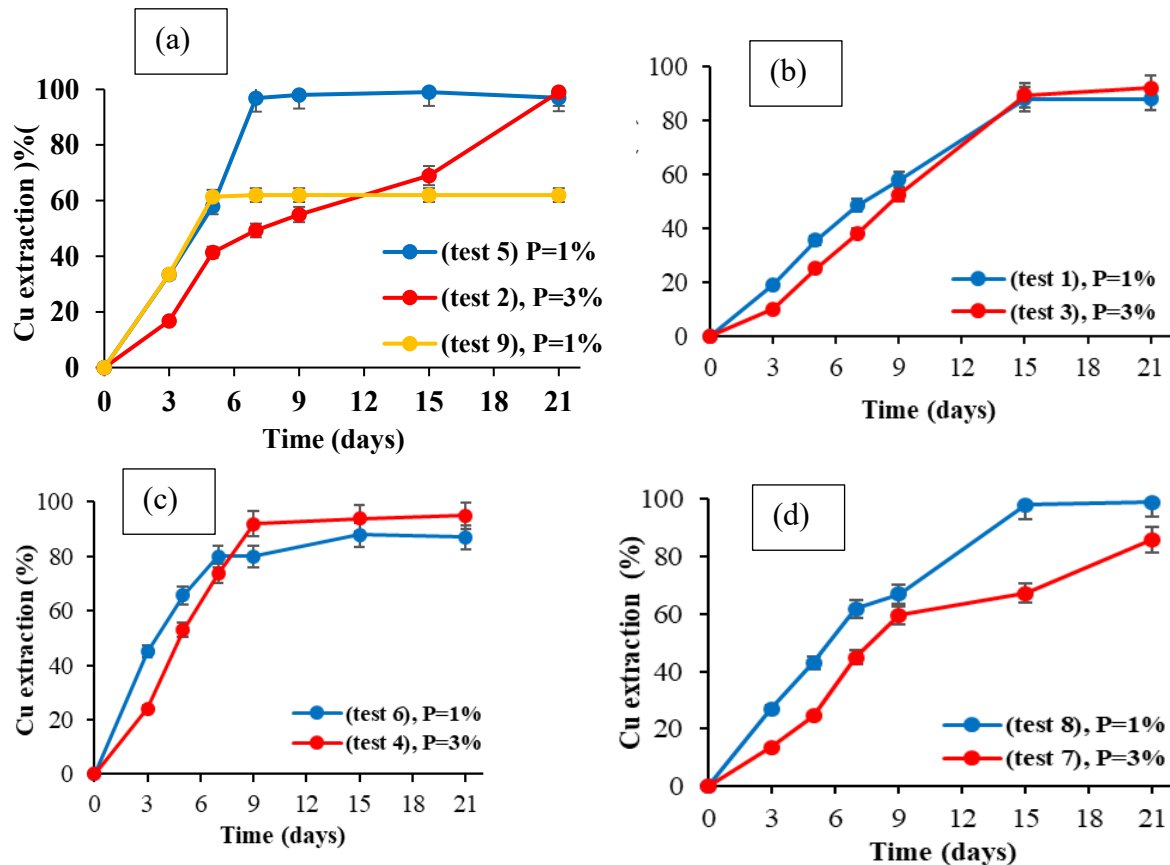


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transfer coefficient because of the low agitation in the shaker, especially oxygen transfer coefficient, was another factor for decreasing copper dissolution [23].



**Figure 1.** Cu extraction (%) from copper sulfide concentrate in 1% and 3% solid. Experimental conditions: a) 0.5 mol/L NaCl and pH 1.5 b) 0.5 mol/L NaCl and pH 2 c) 1 mol/L NaCl and pH 1.5 d) 1 mol/L NaCl and pH 2.

## CONCLUSION

The main conclusions from this study are as follows: 1) During bacterial adaptation with different concentrations of chloride ions at pH 1.5, increasing chloride ions concentration reduced the bacterial count but did not prevent the bacteria from reproducing. 2) Increasing pH in the bioleaching from 1.5 to 2.0 decreased copper dissolution. Therefore, pH=1.5 was more effective for Cu extraction than pH=2.0. 3) The inoculation of bacteria to the leaching process of copper sulfide concentrate with pH=1.5, 1% solid, and 0.5 mol/L NaCl increased copper dissolution from 62% to 98% after 9 d. 4) In bioleaching copper sulfide concentrate, high concentrations of chloride ions (1.0 mol/L) can reduce jarosite formation if solution ORP is controlled. 5) Despite the formation of jarosite and elemental sulfur, copper sulfide concentrate dissolution using *Sulfolobus acidocaldarius* bacteria in the presence of chloride ions (0.5 mol/L

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NaCl) reached approximately 100% after 14 d, which indicated the important role of chloride ions in bioleaching copper dissolution. Less jarosite formed in the bioleaching compared to the leaching process. 6) Higher solid content (3%) requires more time for Cu extraction in leaching and bioleaching processes than low solid content (1%) reported by most researchers. 7) Generally, the bioleaching process for dissolution of copper sulfide concentrates in the presence of chloride ions is efficient at the Midouk Copper Complex in Shahr-e-Babak City. Therefore, using underground water (or piped seawater) can be a suitable option in the bioleaching process of copper extraction.

**Keywords: bioleaching; Sulfolobus acidocalarius; copper sulfide concentrate; chloride ion.**

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# Oral Presentations



## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Biosynthesis of carbon nanoparticles from pine fruit soot and investigation of its physical, antibacterial and cytotoxic properties

Afsaneh Molamirzaei<sup>1</sup>, Zarindokht Emami-Karvani<sup>1\*</sup>, Samaneh Sedighi-Khavidak<sup>2</sup>, Maryam Mohammadi-Sichani<sup>1</sup>, Monir Doudi<sup>1</sup>

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Green synthesis of nanoparticles is simple and biocompatible and can be produced by a wide range of microorganisms and plants. Since the occurrence of drug resistance and its side effects in the treatment of infections have increased in recent years, the aim of this study was to investigate the antibacterial properties and toxicity of carbon nanoparticles synthesized from pine fruits on human skin fibroblast cell line (CRL-2522).

### MATERIALS AND METHODS

Green synthesis of carbon nanoparticles from pine fruits was performed by burning and crystallization of soot. The morphology and characteristics of the nanoparticles were investigated using scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDAX), respectively. Multi Drug Resistance (MDR) bacteria isolated from a clinical laboratory in the city of Isfahan were analyzed and classified. MIC determination and agar disc diffusion test was used to investigate the antibacterial activity of the synthesized carbon nanoparticles against five MDR (Multi Drug Resistance) bacterial isolates that cause urinary tract infections. Additionally, colorimetric MTT technique was used to examine the cytotoxic effect of the produced carbon nanoparticles on healthy human skin fibroblast cells (CRL-2522).

### RESULTS AND DISCUSSION

The synthesized nanoparticles were spherical and according to the morphological findings, had an average size of less than 100 nm. The results of EDAX test revealed that carbon made up 87.08% of the produced nanoparticles. 64 samples of the 100 bacterial isolates responsible for urinary tract infections were found to be resistant to more than three popular antibiotics and belonging to five different bacterial genera. The highest inhibitory effect was on *Escherichia coli* isolate at MIC concentration of 0.25 mg/ml, and the lowest inhibitory effect was on *Klebsiella pneumoniae* isolate. The findings of MTT test demonstrated that the synthesized carbon nanoparticles did not exhibit cytotoxicity to the cell line CRL-2522 at concentrations less than 0.4 mg/ml for 24 hours.

### CONCLUSION

The results of this research showed that carbon nanoparticles synthesized from pine fruit, in addition to antibacterial effect, had no toxicity on human skin fibroblast cells in concentrations less than 0.4 mg/mL. These nanoparticles have the potential to be considered in clinical research in the treatment of bacterial infections.

**Keywords:** Green synthesized, Multi Drug Resistance, Nanocarbon, Urinary tract infection

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### One-Year Surveillance of SARS-CoV-2 RNA in groundwater in Tehran, Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The highly infectious and transmissible Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused a pandemic of acute respiratory disease known as COVID-19, which poses a serious hazard to human health. Although the SARS-CoV-2 RNA has been detected in wastewater from various regions in various countries, there is still not much data available about the frequency of the virus in water sources, particularly groundwater. The purpose of this study was to look for SARS-CoV-2 genome in groundwater samples of Tehran, Iran.

#### MATERIALS AND METHODS

Groundwater samples were collected seasonally from 12 different locations over the course of a year (2021-2022). Firstly, an adsorption-elution concentration method was tested followed by RNA extraction. Afterward, reverse transcription-real time polymerase chain reaction (RT-qPCR) was used to detect the SARS-CoV-2 E and S genes.

#### RESULTS AND DISCUSSION

The RT-qPCR amplification of the E and S genes allowed the detection of SARS-CoV-2 in 2.08% (1/48) of the groundwater samples that were processed by the mentioned viral concentration method.

#### CONCLUSION

Given these results, as well as direct discharge of non-treated or inadequately treated wastewater into groundwater, poor sanitation and leaks in underground pipelines, it warns governments to have more control and oversight over the performance of urban and local treatment plants and water distribution systems.

**Keywords:** SARS-CoV-2, COVID-19, Groundwater, RT-PCR, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Inhibition of *Acinetobacter baumannii* adhesion to human lung epithelial cells by antibody produced against Omp34

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Acinetobacter baumannii* causes infections such as meningitis, bloodstream infections, pneumonia, urinary tract and skin infections in hospitals, especially in intensive care units. The interaction between *A. baumannii* and human lung cells leads to infection and cell death due to adhesion and invasion of these cells. Therefore, it is important to study the interaction of *A. baumannii* with human lung host cells. Outer membrane protein 34 (Omp34) is one of the virulence factors of *A. baumannii*. Cytotoxicity, adhesion, and attack on human epithelial cells, as well as induction of apoptosis and inhibition of autophagy in human cells, are among the functions of this main pathogenic agent. *In-vitro* cell culture methods are a useful tool for investigating the interactions between human epithelium and pathogens that occur during infection because bacterial adhesion to cells is considered to be the first major step in bacterial pathogenesis. In this study, *A. baumannii* ATCC 19606 and a clinical isolate, *A. baumannii* 58ST were analyzed in terms of adhesion and internalization to A549 cell line.

### MATERIALS AND METHODS

rOmp34 was expressed, purified, and injected into groups of BALB/c mice. The antibody titer was measured by the indirect ELISA. Adhesion and internalization of *A. baumannii* strains were studied in A549 cell line.

### RESULTS AND DISCUSSION

Omp34, a conserved and potent immunogen of *A. baumannii*, significantly inhibited adhesion to and internalization in A549 cells of *A. baumannii* strains.

### CONCLUSION

Antibodies raised to Omp34 prevent bacterial adhesion and invasion of A549 lung cells.

**Keywords:** *Acinetobacter baumannii*, Omp34, A549, Adherence

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# Isolation and identification of specific lytic bacteriophage vB\_CacS-ZE against *Cutibacterium acnes* from skin lesions

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## ABSTRACT

### Background and Objectives:

In recent years, prevalence of antibiotic resistance is reported for *Cutibacterium acnes*. The aim of this study was to isolate a specific bacteriophage against *Cutibacterium acnes* to be used for bacteriophage therapy of acne vulgaris caused by this bacterium.

### Materials and Methods:

In this study, samples were collected from different areas of the skin surface of 50 patients aged 18 to 60 years old having symptoms of acne vulgaris. Then, differential diagnostic tests were performed. Two hundred volunteers aged 18-60 years old with healthy skin and free of typical acne lesions were selected for isolation of the bacteriophage. Bacteriophage characteristics such as morphology, stability in different temperature, pH and soluble salts were evaluated. Moreover, adsorption rate, latent and rise periods, burst size and phage genome size estimation using enzymatic digestion was studied.

### Results and Discussion:

TEM analysis showed that the isolated phage was categorized in the *Caudoviricetes* class and named as vB\_CacS-ZE. This bacteriophage is stable in a wide range of temperatures (+4 to +45), pH 4-9 and different salt concentrations (1, 5, 10, 20%). MOI 0.1 was the best phage concentration to inhibit *C. acnes*. In this regard, after 25 min, 86% of vB\_CacS-ZE phages were attached to their hosts, latent and the rise periods were 18 and 12 h, respectively, and its burst size was about 100 phage particles for each infected cell. The bacteriophage genome size was estimated to be about 28,600bp.

### Conclusion:

As a result, if phage therapy can be used to treat skin acne due to the high prevalence of acne vulgaris, the use of local and systemic antibiotics and, accordingly, the cost of treatment will be reduced in the era of antibiotic resistance.

**Keywords:** *Cutibacterium acnes*, antibiotic resistance, acne vulgaris, phage therapy

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# ST-8881 as a predominant clone of ESBL-producing *E. coli* isolated from nosocomial infection, healthcare worker and sewage in a tertiary hospital

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

*Escherichia coli* (*E. coli*) is a prevalent bacterial pathogen responsible for various infections. Human populations, particularly hospital health workers, can serve as reservoirs for *E. coli* and facilitate its transmission through fecal contamination and inadequate hygiene practices. This study aims to investigate the involvement of health workers in transmitting infections caused by ESBL-producing *E. coli* within a tertiary hospital setting. This study aims to assess the contribution of health workers to the transmission of ESBL-producing *E. coli* infections in a tertiary hospital, shedding light on their role as potential sources of nosocomial infections.

### MATERIALS AND METHODS

In this study conducted from June 2021 to October 2021, traditional culture methods were employed to isolate 371 *E. coli* strains from the fecal samples of health care workers, hospitalized patients, and hospital wastewater. Among these isolates, 75 were identified as ESBL-producing *E. coli*. Further analysis revealed that out of the 30 ESBL-producing *E. coli* isolates selected, six were derived from hospital wastewater, twelve from hospitalized patients, and twelve from health care workers. These isolates were subsequently subjected to MLST analysis.

### RESULTS AND DISCUSSION

MLST analysis of the selected ESBL-producing *E. coli* isolates revealed that the predominant clone identified was ST8881, which was found in isolates from health care workers, hospital sewage, and patients hospitalized in the neurology and ICU B ward. Furthermore, four strains from hospitalized patients in the orthopedic departments and men's surgeries were identified as ST320.

### CONCLUSION

Given the high incidence of ESBL-producing *E. coli* infections and the potential transmission cycle involving hospital health personnel and hospitalized patients, implementing changes in hospital infection control policies can play a crucial role in reducing these infections.

**Keywords:** *E.coli* , ESBL , *Escherichia coli* , health workers , MLST

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antioxidant, anticancer, and antibacterial effects of epsilon-poly-L-lysine produced by novel *Bacillus* strain

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## Abstract

### BACKGROUND AND OBJECTIVES

Epsilon-Poly-L-lysine ( $\epsilon$ -PL) is a natural cationic homopolypeptide consisting of at least ten lysine residues linked together by peptide bonds between the  $\alpha$ -carboxyl and the  $\epsilon$ -amino groups. The  $\epsilon$ -PL is biodegradable, water-soluble, edible, and non-toxic to humans and the environment with heat stability and unique structure characterization which have made it a crucial biopolymer in medicine and industries. This cationic biopolymer and its derivatives offer a wide range of applications such as food preservatives, dietary supplements, biodegradable fibers, drug carriers, gene carriers, and anticancer enhancer agents. Since the first report of  $\epsilon$ -PL production by *Streptomyces albulus* 346 as an extracellular product, several  $\epsilon$ -PL producer microorganisms such as *Streptomyces noursei*, *Streptomyces diastatochromogenes*, *Kitasatospora kifunense*, and *Bacillus subtilis* have been introduced so far, but the identification of novel strains with higher production rate is still ongoing. In addition, due to its efficient functional groups and positive charge,  $\epsilon$ -PL can be a potent microbial candidate as an antimicrobial, antioxidant, and anticancer agent.

### MATERIALS AND METHODS

In this study, we isolated and identified *Paenibacillus polymyxa* HS6 (accession number: MW791431) from soil samples as a novel  $\epsilon$ -PL producer with a maximum yield of 1.801 g.L<sup>-1</sup>. Furthermore, the antibacterial activity, antioxidative capacity, and cytotoxicity effects of  $\epsilon$ -PL produced by isolate were examined. To obtain pure cationic polypeptide, chemical precipitation with sodium tetraphenylborate (NaTPB) was used. The purified cationic compound was confirmed to be  $\epsilon$ -PL by High-Performance Liquid Chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), <sup>13</sup>C nuclear magnetic resonance (<sup>13</sup>C NMR), and Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE). The antioxidant activity of the purified  $\epsilon$ -PL was then determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Moreover, the anticancer and cytotoxic effects were evaluated against MCF-7, HT-29, and L929 cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and flow cytometry. In addition, the antibacterial activity of  $\epsilon$ -PL was evaluated against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Serratia marcescens* ATCC 13880, and *Klebsiella pneumoniae* ATCC 13883 by microdilution method.

### RESULTS AND DISCUSSION

The maximum yields of 1.8 g/l  $\epsilon$ -PL were obtained by *P. polymyxa* HS6 after 20 hours of incubation in M3G culture medium. The results showed that the antioxidant capacity of  $\epsilon$ -PL was concentration-dependent, and there is a significant correlation between concentration and radical scavenging activity.

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Radical scavenging activity of  $\epsilon$ -PL at a minimum concentration (0.18 mg/mL) was at least 7% which increased to 90% at 8 mg/mL. Furthermore, the highest anticancer activity was observed against the MCF-7 cell line (99.5%) at 0.5 mg/mL concentration, while almost no toxicity was recorded towards L929 cells. Also, the results showed that  $\epsilon$ -PL in less than 2 mg/ml has a great antibacterial effect and can inhibit the growth of the bacteria.

### CONCLUSION

Despite tremendous medical and pharmaceutical research progress, combat with cancers and infections is still one of the most global public health challenges. Some of the natural compounds with hopeful bioactive properties represent possible candidates to overcome the disadvantages and side effects of chemical and synthetic agents. Based on the results obtained in this study, it is suggested that the  $\epsilon$ -PL produced by *P. polymyxa* HS6 is a potential bioactive compound with significant anticancer, antioxidant, and antibacterial properties.

**Keywords:** Antimicrobial peptide, Epsilon-poly-l-lysine, Anticancer agent, Bioactive compound

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Microbial profiling of *Paederus fuscipes* (Coleoptera: Staphylinidae) through Next-Generation Sequencing

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Paederus fuscipes* is the most famed beetle, which causes dermatitis or conjunctivitis in humans via discharging pederin toxin. Pederin with antitumor and antiviral properties have been shown to be synthesized by uncultured *Pseudomonas*-like endosymbionts. This study was aimed to characterize the structure and diversity of *P. fuscipes* microbial communities.

#### MATERIALS AND METHODS

Adult rove beetles were gathered from June–July 2019–2020 from the humid areas of Guilan, Mazandaran, and Golestan provinces where highest cases of linear dermatitis have been reported. The Illumina HiSeq sequencing platform was used to determine the bacterial diversity of the 16S rRNA gene (V3-V4) in insect samples in terms of gender, organ, and location.

#### RESULTS AND DISCUSSION

The OTUs identified from *P. fuscipes* specimens were collapsed into 40 phyla, 112 classes, 249 orders, 365 families, 576 genera, and 106 species. Thirty top genera made up > 94% of the *P. fuscipes* microbiome, with predominating *Pseudomonas*, followed by the *Spiroplasma*, *Apibacter*, *Enterococcus*, *Dysgonomonas*, *Sebaldella*, *Ruminococcus*, and *Wolbachia*. *Spiroplasma* / *Apibacter* as well as *Pseudomonas* / *Pseudomonas* were the most abundant in the genitals / intestines of male and female beetles, respectively. Additionally, male and female rove beetles were characterized by distinctive microbiota in different organs, likely reflecting different functions and/or adaptation processes.

#### CONCLUSION

These findings may eventually lead to ecological insights into the production and utilization of defensive compound of pederin as well as the management of linear dermatitis with the use of available antibiotics.

**Keywords:** *Pseudomonas*-like, *Paederus fuscipes*, *Dermatitis linearis*, Pederin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Optogenetic a new tool in bacteria controlling gene expression as a switch on/off

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Being able to control microorganisms with a switch that turns them on or off is like a dream. Optogenetics is a technique that uses light to regulate gene expression. A specific wavelength can activate specific proteins that are sensitive to light, and these photoreceptors can control gene promoter, activate signaling cascades and affect cell function in various ways. This method allows researchers to monitor their activity in real time, enabling them to exert fine control over which genes turn on or off, or when they activate. Compared to the traditional methods of gene expression control, it offers several advantages, such as high spatial and temporal precision and reversibility. Optogenetics has many potential applications, such as the production of therapeutic proteins, the study of disease processes, the development of new treatments for genetic disorders, or regulating the virulence of microbial pathogens because of antibiotic resistance, which is a serious threat to health. Regulate microbial metabolite production in fermentations or culture media. This article summarizes the mechanisms, design principles, applications, advantages and limitations of optogenetics in bacteria.

From the 1970s to 1976, the impact of light on cell and gene function was discovered by scientists, which led to the development of optics. In 2005, Levskaya et al. first developed optogenetics, a technique that allows the control of red light-controllable LacZ expression in *E. coli*. Optogenetics has proven to be an effective tool in managing diseases like epilepsy, controlling pathogen severity, and managing septicemia. Recently, blue light wavelengths have been used with endoscopy to control the pathogenicity of *Helicobacter*.

#### MATERIALS AND METHODS

Scientific papers reviewed.

#### RESULTS AND DISCUSSION:

The placement of engineered photoreceptors upstream of promoters or binding to proteins can have important implications in cancer treatment. Researchers have been able to manage diseases and control pathogens by using photoreceptor proteins as auto-inducers.

#### CONCLUSION

Optogenetics is simpler CRISPR for manipulating genes and cells.

**Keywords: Optogenetic, on/off switch, Photoreceptors, Gencontrolling, Microbial metabolites, Antibiotic resistance.**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Study of the antibacterial efficacy of tetracycline-conjugated gold nanoparticles

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The emergence of antibiotic-resistant bacteria has become a growing concern in healthcare and necessitates the development of novel strategies to combat these pathogens. In this study, we investigate the antibacterial activity of tetracycline conjugated gold nanoparticles (Tc-Au NPs). Gold nanoparticles have gained interest due to their unique physicochemical properties, including their ability to efficiently deliver drug payloads.

### MATERIALS AND METHODS

In this study, we conjugate tetracycline, a broad-spectrum antibiotic, onto the surface of gold nanoparticles to improve its efficacy against bacterial pathogens. Formation of nanoparticles was determined using visible-UV spectrophotometer, Fourier-transform infrared spectroscopy, and transmission electron microscopy. The Mueller-Hinton broth environment was used for minimum inhibitory concentration (MIC) inhibition of Tc-Au NPs.

### RESULTS AND DISCUSSION

Our results demonstrate that Tc-Au NPs display enhanced antibacterial activity compared to free tetracycline, with lower minimum inhibitory concentrations observed. Additionally, the cytotoxicity of Tc-Au NPs is evaluated using in vitro cell viability assays. The results reveal that Tc-Au NPs exhibit low cytotoxicity, indicating their potential as a safe and effective antibacterial therapy.

### CONCLUSION

Our findings provide valuable insights into the potential use of tetracycline conjugated gold nanoparticles as an alternative antibacterial therapy with improved efficacy and safety profiles. Overall, this study contributes to the ongoing efforts in combating antibiotic resistance and offers a promising platform for the development of nanoparticle-based antibacterial agents.

**Keywords:** Tetracycline antibiotics, gold nanoparticles, synthetic approach, antibacterial activity.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular typing of *Acinetobacter baumannii* complex isolated from clinical samples in west of Iran

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## Abstract

### Background and Objectives

*Acinetobacter baumannii* is a multi-drug resistant gram-negative organism. Due to the increasing number of *A. baumannii* infections in hospitals, molecular genotyping can provide information to identify the principles of the epidemiological spread of this microorganism. The present study was conducted to investigate the antibiotic resistance of *A. baumannii* complex clinical strains isolated from intensive care units (ICUs) of hospitals in Sanandaj and Kermanshah cities and to determine the genetic relationships between isolates.

### Material and Methods

In this cross-sectional study, 75 *A. baumannii* complex strains were isolated from various clinical specimens of patients hospitalized in the ICUs of three hospitals in Sanandaj and Kermanshah, in west of Iran during six months. The antibiotic susceptibility was determined by the disk diffusion agar method. Metallo-beta-lactamase producing strains were identified using the PCR method. ERIC-PCR and Multilocus Sequence Typing (MLST) were used to determine the genetic relationships between isolates. The nucleotide sequences of seven housekeeping genes were analyzed in the Pasteur Pub-MLST database and the genetic relationships of the isolates were determined.

### Results and Discussion

The highest level of resistance was found to Cefotaxime (99%), Ciprofloxacin (89%), Meropenem (88%), and Gentamicin (87%). The lowest resistance was found to Tetracycline (59%). A total of 89% of the strains was multidrug-resistant (MDR). PCR indicated that 65 isolates (87%) carried the *bla*<sub>VIM</sub> gene, while none of the isolates carried *bla*<sub>SIM</sub>, *bla*<sub>GIM</sub>, *bla*<sub>NDM</sub>, *bla*<sub>IMP</sub> and *bla*<sub>SPM</sub>. ERIC typing and MLST showed a clonal spread of isolates in the hospitals in Sanandaj and Kermanshah. The sequence type (ST) 415 was obtained.

### Conclusion

This study demonstrated a high level of resistance and clonal dissemination of *A. baumannii* isolated from patients hospitalized in the ICUs of hospitals in Sanandaj and Kermanshah. The increasing prevalence of hospital infections and the emergence of resistance among *A. baumannii* isolates highlight the need to investigate the genetic diversity between isolates. The present study can aid in developing methods to control the spread of this pathogen.

**Keywords:** *Acinetobacter baumannii*, Metallo-beta-lactamase, Antibiotic resistance, *bla*<sub>VIM</sub> gene, Multilocus sequence typing

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The prevalence of *Leptospira* infection in abortions of small ruminants by PCR

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Leptospirosis is a zoonosis caused by pathogenic spirochetes of the genus *Leptospira*. The disease is maintained in nature by chronic renal infection of carrier animals and acquired by direct or indirect contact with urine or tissues from infected animals. Leptospirosis affecting most of small ruminants (goats, sheep) in which it causes several signs including abortions, stillbirths and mummification.

The aim of this study was to determine the prevalence of leptospirosis in small ruminant by PCR based on *lipL32* gene in IRAN

#### MATERIALS AND METHODS

Renal tissue samples were collected from 50 abortions of small ruminants with clinically suspected leptospirosis attending the department of Microbiology, Razi Vaccine & serum Research Institute, Karaj during the period of April 2019 and March 2023. The prevalence of leptospiral infection in specimens of aborted small ruminants was studied by PCR based on *lipL32* gene. Statistical methods were performed to determine the significant association of various demographic data using the statistical software SPSS.

#### RESULTS AND DISCUSSION

A total 7 (14%) out of 50 renal samples were positive by PCR. Statistically significant association was found between the number of pregnancies and leptospiral infection. Although age and sex of abortions had no significant association with leptospiral infection.

#### CONCLUSION

Regarding the high prevalence of abortion caused by pathogenic leptospire in this study (14%), it is necessary to boost the information about the prevalence of leptospiral infection in different regions of our country.

**Keywords:** Leptospirosis, *Leptospira*, Small ruminants, PCR, Serovars

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### A new approach in food packaging: smart, active, biodegradable nanobiocomposite

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#### ABSTRACT

The change and diversity of food production and breeding systems, change in lifestyle, increase in tourism and immigration along with the increase in global food trade have made the risk of foodborne diseases even more evident. The lack of food resources, the idea of storing and increasing shelf life of food storage, have encouraged researchers to find a way to improve food quality, long-term storage and reduce the value of food products. Therefore, new studies are focused on the development of new methods, techniques, and procedures in order to processing, packaging, and implementing quality control of food as well as the supply system of food products. Meanwhile, food packaging is one of the most effective approaches to achieve this goal. Food packaging in the food chain to facilitate transportation, flexibility, improve shelf life, organoleptic characteristics and reduce physicochemical changes (moisture, Color, taste, weight and texture (bioavailability), as well as food protection against microbial and chemical contamination are used. According to the research of the researchers, smart packaging containing indicators (O<sub>2</sub> and CO<sub>2</sub> detectors, pH indicators (goodness, temperature and time) sensors, as well as biosensors of pathogenic bacteria have been introduced to detect the freshness of food products. Using the pH indicator in the form of a smart packaging (in the form of a modified atmosphere, time-temperature changes) and following the creation of volatile nitrogen compounds during food spoilage, to consumers It makes it possible to distinguish between fresh and spoiled food without opening the package. The use of natural antimicrobial compounds ( extracts and essential oils of medicinal plants) in smart packaging can increase the shelf life of food by inhibiting foodborne pathogens or delaying the changes of microbial and chemical spoilage. Biopolymers (protein, carbohydrates and lipids or combination of them) are used to prepare biodegradable film for food packaging and should be renewable, cheap , produced from waste and as a coating or film on the material. The use of nano properties through the improvement of the materials used in packaging causes the development of technologies for designing methods and producing safe and degradable food packaging for the environment, which increase shelf life and freshness of food products.

**Keywords: Food packaging, Smart, Sctive, Biodegradable, Nanobiocomposite**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The distribution of *Bartonella* spp across the countries in the WHO Eastern Mediterranean Region (WHO-EMRO)

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Bartonella* is a Gram-negative, facultative intracellular bacterium that can cause vector-borne diseases in humans, particularly in immunocompromised individuals. The aim of this study was to describe the geographical distribution of different *Bartonella* spp in the countries of the WHO Eastern Mediterranean Region (WHO-EMRO). However, there is limited data available on the distribution of *Bartonella* species, as well as the status of its reservoirs, vectors, and human cases worldwide

#### MATERIALS AND METHODS

In this study we searched of published reports and studies on *Bartonella* species in WHO-EMRO region countries. This search was conducted using different databases until August 2022. Finally, we classified and reported on the status of human cases, reservoirs, and vectors associated with each *Bartonella* species in different countries the region.

#### RESULTS AND DISCUSSION

Only 13 of the 22 WHO-EMRO countries had reports of *Bartonella* infection. In WHO-EMRO countries, fifteen distinct *Bartonella* species have been detected including *Bartonella henselae*, *Bartonella quintana*, *Bartonella elizabethae*, *Bartonella clarridgeiae*, *Bartonella vinsonii*, *Bartonella doshiae*, *Bartonella rochalimae*, *Bartonella tribocorum*, and *Bartonella koehlerae*

#### CONCLUSION

The results of this study show that *Bartonella* infection is very important in EMRO countries, but neglected by doctors and health care systems.

**Keywords:** *Bartonella*, Bartonellosis, WHO-EMRO, zoonosis.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Efficient ethanol production of an industrial strain of *Saccharomyces cerevisiae* with improved ethanol tolerance via evolutionary engineering

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

In order to optimize existing ethanol production, it is essential to maintain *Saccharomyces cerevisiae* growth and sugar metabolism during the fermentation process. Yeast viability and vitality in high-stress fermentation conditions are significant parameters in reaching the goal. Ethanol stress, as the main stress factor that causes a decrease in ethanol production in fermentation, causes many changes in *S. cerevisiae*, which include increased inhibition of metabolism, decreased growth and absorption of nutrients, and disruption of the membrane structure. It leads to an increase in membrane permeability and electrochemical loss. Ethanol tolerance is a complex phenotype that can be affected by diverse alleles that may show complex interactions and which may vary between different strains. Evolutionary engineering has considerable industrial consequences due to the toxicity that many bio-renewables have on microorganisms through production capacity and viability.

### MATERIALS AND METHODS

In the present study, to improve the ethanol tolerance phenotype in a strain isolated from the fermenter of an alcohol factory. The mother strain was mutated by physical and chemical methods. Mutants were screened using medium containing 1-butanol. The original parent and the mutants were evolved over 144 days by evolutionary engineering strategy, while the ethanol production of the selected strains was investigated. Ethanol production of selected strains was investigated in laboratory and industrial fermenters.

### RESULTS AND DISCUSSION

According to the increase in the maximum growth rate, 96 strains were selected including parental strain and mutants, and the amounts of ethanol production of these strains were evaluated after evolutionary adaptation tests. Ethanol production of F121, which was mutated with EMS before the adaptive evolution test and then evolved at 9% v/v ethanol, was improved from  $96.0 \pm 0.04$  g/L to  $109.3 \pm 1.1$  g/L.

### CONCLUSION

Nowadays, evolutionary engineering is considered a powerful method in creating industrial strains with a desired phenotype. In this research, in order to increase ethanol tolerance in an industrial strain, evolutionary engineering strategy was used. To increase initial genetic diversity the population, before starting adaptive evolution experiments, mutagenesis was performed, and the results showed that mutagenesis accelerated the process of reaching the desired phenotype by increasing genetic diversity. The strain developed on an industrial scale was able to increase the yield and lead to an increase in efficiency.

**Keywords:** *Saccharomyces cerevisiae*, ethanol production, ethanol tolerance, evolutionary engineering

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Potential of renewable sustainable energy to save the Planet

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Energy is the entrusting source for various man activates and dawn the history its main source has improved from wood to coal and crude petroleum to gas and very recently to renewable. The fossil fuels like coal and petroleum are limited, which are relatively safer to handle, process, transport and utilize. However, based on the type of end use like combustion or using to generate safer energy emits Green House Gases (GHG). Greenhouse gases including CO<sub>2</sub>, methane, refrigerant gases and so on since industrial revolution had adverse effect on climate changes including the earth warming (approximately 2 °C) and souring oceans pH. Further, GHJG have impaired human health as a result of increased rate of people loosing life, in particular those suffering from hearth and lungs complications in addition to old age. Furthermore, fortified acid rains, and enhanced drought at one corner and tsunami at other. The highest share among the six most undesirable GHG goes to CO<sub>2</sub> and methane emissions which is about 56 and 25%, respectively. The reduction of emitted greenhouse gas (GHG) and atmospheric pollutants are constituted as the foremost energy and environmental policy universally. The most polluting nation after China are USA, India, Russia, Japan, Germany, Iran, Canada, South Korea and The United Kingdom. The aforementioned nations are signatory to the United Nations Framework Convention on Climate Change (UNFCCC) and the head of the states have attended various protocols including Kyoto, Copenhagen, Paris and many more. However, practical achievements documented so far are in limit, where few nations are trying hard to reach the needful. However renewable energies like, solar, wind, geothermal, hydropower, ocean, and bioenergy are potential source to change the global scenario to some extent. Iran's standing would be addressed. Furthermore, energy sources with lesser carbon molecules than gasoil at preset are preferable. Immediate future fuels opt for a type without carbon molecules. Hydrogen is a desired candidate to replace the fossil fuels. Hydrogen currently in large scale is produced by steam reforming of naphtha, followed by hydrolysis of water, biomass and bacteria. Biohydrogen produced by dark fermentation is more advantageous as result of higher rate of production.

### MATERIALS AND METHODS

Have used my earlier publications including papers, students thesis, books and related published papers.

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### RESULTS AND DISCUSSION

Man has been using carbon based materials down the history for various purposes, which has emitted Green House Gases (GHG) and the rate of pollution being enhanced by socioeconomic improvements since industrial revolution. GHG trap gases and result in pollution, acid rain, global warming leading to climate changes. The universal approach is to reduce burning and combustion of fossil fuels of high carbon number. However there is enough concern to switch to the least or no-carbon based fuels like from solar, wind, ocean, geothermal, hydropower and bioenergy. Bioenergy stands at the forefront of technological improvements to fulfill the universal requirements.

### CONCLUSION

There is global awareness and fundamental need to decrease the rate of climate changes including global warming by the least or non-polluting energy source. The emission gases should not accumulate GHG to change the planet atmosphere for cleaner air, blue sky and better place to live. At present fuels with less carbon is preferred and platform for no-carbon fuel like renewable sustainable hydrogen is in progress.

**Keywords:** Sustainable, renewable energy, solar energy, biohydrogen

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Genotypes, immune escape and drug resistant mutations of Hepatitis B virus among isolates of Afghanistan

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Hepatitis B virus (HBV) infection remains a significant public health concern worldwide, leading to severe liver diseases such as chronic hepatitis, hepatocellular carcinoma, and liver cirrhosis. It continues to be prevalent among high-risk populations and non-immunized individuals in regions with high endemicity. Afghanistan is no exception, but there is limited information about the molecular aspects of HBV circulating in the country. This study aimed to investigate the genotyping patterns, mutations, and other molecular characteristics among HBV isolates in Afghanistan.

### MATERIALS AND METHODS

This cross-sectional study included patients who tested positive for HBsAg. Approximately 5 ml of venous blood was collected, and the separated serum was serologically tested for anti-HBc, HBsAg, anti-HBs, and HBeAg. Molecular examinations, including DNA extraction and various PCR assays, were performed to determine HBV genotypes. Additionally, the full S gene was amplified, sequenced, and analyzed phylogenetically to investigate mutations and genotyping of HBV.

### RESULTS AND DISCUSSION

Out of 120 cases, all were positive for HBsAg. The mean age of the patients was  $39.2 \pm 5.1$  years, ranging from 18 to 75 years, and 38% were male. Phylogenetic analysis revealed that all isolates belonged to the HBV genotype D. Sub-genotype distribution indicated a predominance of D1, with 3.9% classified as D2. The most prevalent mutations observed were N202S and T208I. Furthermore, immune escape mutations, including I110L (16%), P120S (8.7%), and S143L (25.8%), were found in polymorphic sites of HBsAg. Within the YMDD motif, M204V and D205T mutations were present in 5.3% of cases.

### CONCLUSION

The genotyping pattern of HBV in Afghanistan is consistent with neighboring countries and regions. Further investigation is required to trace clades and conduct evolutionary analysis to determine the main routes of transmission and circulation. The incidence of viral escape and drug-resistant mutations was noteworthy, necessitating additional research.

**Keywords:** Hepatitis B virus, HBV, HBsAg-positive, Phylogenetic analysis, mutation, Afghanistan

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the frequency of bacteriocin-producing *Enterococcus faecalis* and its correlation with *gelE*, *cylA*, and *esp* genes isolated from hospitalization patients

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Bacteriocins are antimicrobial peptides (AMPs) and many lactic acid bacteria (LAB) can produce them. The present study aimed to assess investigating the frequency of bacteriocin-producing *E. faecalis* and its correlation with *gelE*, *cylA*, and *esp* genes isolated from hospitalization patients

### MATERIALS AND METHODS

This study was performed on 124 *E. faecalis* isolates. The soft-agar overlay technique was used for bacteriocin production assay. Bacteriocin sensitivity was observed as clear zones of growth inhibition surrounding the producer *E. faecalis* isolates. The presence of *As-48*, *enLA* and *entA* genes encoding of bacteriocins and *gelE*, *cylA*, and *esp* genes encoding of virulence factors were determined by PCR method.

### RESULTS AND DISCUSSION

24.1% (30/124) isolates were bacteriocin-producing *E. faecalis* by the phenotypic method. Bacteriocin-producing *E. faecalis* isolates have inhibitory effect against *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *Staphylococcus aureus* (MSSA), *Acinetobacter baumannii*, *Escherichia coli*, *Listeria monocytogenes*, *Brucella abortus* and *Clostridium difficile*. The frequency rate of *As-48* in bacteriocin-producing *E. faecalis* was 6.6% (2/30), *enLA* was 70% (21/30) and *entA* was 43% (13/30). Of *enLA* positive bacteriocin-producing *E. faecalis*, 4.7% (1/21) were *GelE* positive, 57.14% (12/21) were *cylA* positive, and 85.7% (18/21) were *esp* positive.

### CONCLUSION

The frequency of *enLA* was high in our study. High frequency of virulence factor genes including *cylA* and *esp* may help the pathogenicity of bacteriocin-producing *E. faecalis*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# One-year assessment of Human Sapovirus presence in groundwater in Tehran, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Infection with human sapoviruses (HuSaVs) is a global public health concern that contributes significantly to the burden of gastrointestinal disorders by causing acute gastroenteritis in sporadic cases and also outbreaks. For individuals living in rural and remote areas, as well as for their animals, groundwater is a crucial water supply. Furthermore, the microbiological quality of groundwater has become a significant concern for people's drinking water safety and the food security of agricultural products such as vegetables. HuSaVs, including genogroups I, II, IV, and V, cause diarrhea and vomiting in people of all ages. This is the first report of the HuSaVs RNA detection in groundwater in Iran.

### MATERIALS AND METHODS

Seasonally, 48 groundwater samples were collected from 12 sites using the grab sampling method. A virus adsorption-elution concentration method was utilized followed by viral RNA extraction. HuSaV genome was detected using a conventional reverse transcription polymerase chain reaction (RT-PCR).

### RESULTS AND DISCUSSION

HuSaV was found in 2.08% (1/48) of the groundwater samples that were processed using the conventional RT-PCR to amplify the capsid region of the viral genome.

### CONCLUSION

The presence of HuSaVs in groundwater indicates possibility of human fecal contamination, inefficiency in wastewater treatment systems, and probable discharge of treated or untreated wastewater into water sources, which need more effective control on water sources and applying contamination prevention protocols.

**Keywords:** Human Sapovirus, Gastroenteritis, Groundwater, RT-PCR, VIRADEL, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Synergistic Antimicrobial and Antibiofilm Activities of Methanolic and Ethyl Acetate Extracts from *Rosa canina* Fruits in Combination with Tetracycline against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES:

The rise of antibiotic resistance necessitates the exploration of alternative approaches to combatting microbial infections. Natural products have shown potential as adjunct therapies to enhance the antimicrobial efficacy of conventional antibiotics. This study aimed to evaluate the antimicrobial (MIC and MBC concentrations) and anti-biofilm activities of methanolic and ethyl acetate extracts derived from *Rosa canina* fruits against *Pseudomonas aeruginosa* ATCC27853 and *Staphylococcus aureus* ATCC12600, both individually and in combination with tetracycline.

### MATERIALS AND METHODS:

Methanolic and ethyl acetate extracts were obtained from *Rosa canina* fruits using standard extraction techniques. The antimicrobial activity was assessed using the micro broth dilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *P. aeruginosa* and *S. aureus*. Additionally, the anti-biofilm activity was quantified using crystal violet staining method. The extracts were then tested in combination with tetracycline to assess any potential synergistic effects using fractional inhibitory concentration determination (FIC).

### RESULTS AND DISCUSSION

Both the methanol and ethyl acetate extracts from *Rosa canina* fruits exhibited mild antibacterial activity against *P. aeruginosa* and *S. aureus*. The MIC values for *P. aeruginosa* were found to be 8 and 4 mg/mL for the methanol and ethyl acetate extract, respectively. For *S. aureus*, the MIC values was 16 mg/mL for the ethyl acetate extracts. Furthermore, the extracts demonstrated potent anti-biofilm activity, inhibiting biofilm formation and eradicating preformed biofilms. When combined with tetracycline, the extracts exhibited synergistic effects, resulting in enhanced antimicrobial and anti-biofilm activities compared to individual treatments.

### Conclusion

This study highlights the significant antimicrobial and antibiofilm activities of the methanolic and ethyl acetate extracts obtained from *Rosa canina* fruits against *P. aeruginosa* ATCC27853 and *S. aureus* ATCC12600.

**Keywords:** *Rosa Canina*, Synergy, Anti-Biofilm, Antibacterial

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# Isolation and characterization of a lytic bacteriophage against *Klebsiella pneumoniae* and evaluation of its antibacterial effect on clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Due to the emergence of antibiotic-resistant strains of bacteria such as *Klebsiella pneumoniae*, the use of antibacterial potential of bacteriophages has been considered as an effective approach in controlling the infections.

### MATERIALS AND METHODS

After isolating lytic bacteriophage (PKpMa1/19) against *K.pneumoniae* PTCC1290 by agar bilayer method, purification, titration and enrichment of the bacteriophage were performed. Morphological characteristics of PKpMa1/19 were determined using electron microscopy. One step growth, latent period, burst size and lytic activity of the phage in different environmental conditions (temperature, pH, UV and Chloroform) were determined. In the next step, host range of the bacteriophage was evaluated against different clinical isolates from clinical infections using spot test.

### RESULTS AND DISCUSSION

Bacteriophage belonging to *Tectiviridae* family was isolated and purified. Its latent period and burst size were 20-25 min and 311 PFU, respectively. Appropriate lytic effect of the bacteriophage was shown between -22 and 37 °C. Stability of the phage was high in pH= 4-10. Chloroform had no effect on viability of the phage. The phage (PKpMa1/19), was effective against one of the 20 clinical isolates of *K.pneumoniae*, but it showed antibacterial effect on 10 *Pseudomonas aeruginosa* and 16 *Staphylococcus aureus* isolates.

### CONCLUSION

Phage therapy could be a natural biologic approach against clinical isolates of bacteria in nosocomial infections, but evaluation of antibacterial effects of candidate phage(s) against causative bacterial isolates and determining their characteristics is essential for therapeutic application.

**Keywords:** Antibiotic Resistance, Bacteriophage, *Klebsiella Pneumonia*, Phage Therapy

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antimicrobial and cytotoxicity effects of *Lactiplantibacillus pentosus* cell extract on standard and clinically resistant *Pseudomonas aeruginosa* and U87 glioblastoma brain cell line

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nowadays, the use of lactobacillus cell extracts is one of the new strategies, which its biological properties such as antimicrobial, anti-inflammatory, antiviral, anti-immunomodulatory, and anti-cancer activity have been highly regarded. The aims of this study was to investigate the effect of *Lactiplantibacillus pentosus* cell extract and its effect on standard and clinical *Pseudomonas aeruginosa* as well as evaluation its anticancer properties against U87 glioblastoma cell line by MTT assay.

### MATERIALS AND METHODS

In this study, the cytoplasmic extract of *L. pentosus* was prepared by sonication method. The concentration and protein content of extract were evaluated by Bradford method and SDS-PAGE respectively. The antimicrobial property of cytoplasmic extract of *L. pentosus* was investigated by microdilution broth and minimum inhibitory concentration (MIC) was determined. The cytotoxicity effect of bacterial extract was evaluated by MTT assay. Finally, ANOVA and Tukey analysis was done in statistical study.

### RESULTS AND DISCUSSION

The OD of *L. pentosus* cytoplasmic extract was assessed as 0.189 at 600 nm via ELISA reader, and final concentration by Bradford technique was 12.357 µg/µl. The approximated protein content was 18.482%. Based on microdilution test, the MIC of *L. pentosus* extract on clinical *P. aeruginosa* was 1000 µg/ml. ANOVA analysis on MTT results with different concentrations of cytoplasmic extract, cleared that the percentage of cell culture survival reduced as the concentration of cell extract increases and a significant relationship between bacterial concentration and reduced in cell culture survival was found (P Value <0.0001).

### CONCLUSION

In the study of Parvin et al 2022, the antimicrobial and anti-biofilm properties of *L. pentosus* MSCIN-24 and MSCIN-25 against food spoilage and local pathogens was evaluated. They showed glycolipid biosurfactants obtained from these strains had broad antimicrobial activity against local pathogens and food with MIC in the range of 5 to 15 mg/ml and caused cell disruption. Also, Abd Ellatif SA et al 2022, showed the probiotic effect and anticancer activity of *L. plantarum* ESSG1 (MZ683194.1) and *L. pentosus* ESSG2 (MZ683195.1) isolates from dairy products. They revealed that application of these strains will reduce the use of antibiotics in clinical try. In conclusion, in this study, the antibacterial and cytotoxicity effects of *L. plantarum* cell extract has been evaluated on clinical *P. aeruginosa* and U87 cell line is in consistent with a number of recent studies.

**Keywords:** *Lactiplantibacillus pentosus*, *Pseudomonas aeruginosa*, U87 Cell line

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The anti-biofilm effects of *Lactobacillus plantarum* and *Lactobacillus reuteri* cell-free supernatant on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Staphylococcus aureus* and *Pseudomonas aeruginosa* are important infectious agents in humans worldwide. They are associated with antibiotic resistance, adhesion to surfaces, biofilm formation on medical instruments, and the development of chronic opportunistic infections. These factors pose significant challenges in medicine, necessitating the search for alternative therapeutic agents. One of these solutions is direct use of natural antimicrobial producers, such as probiotics. This study aimed to describe the inhibitory activity of lyophilized cell-free supernatants (CFS) of *Lactobacillus plantarum* PTCC 1745 and *Lactobacillus reuteri* PTCC1655 against *P. aeruginosa* and *S. aureus*. One potential solution is the use of natural antimicrobial producers, such as probiotics. This study aimed to investigate the inhibitory activity of lyophilized cell-free supernatants (CFS) of *Lactobacillus plantarum* PTCC 1745 and *Lactobacillus reuteri* PTCC1655 against *P. aeruginosa* and *S. aureus*.

#### MATERIALS AND METHODS

Antibacterial activities of the CFS of lactobacilli were assessed by agar well diffusion and microtiter plate method to obtain MIC and MBC. The characteristics of the antibiofilm were analyzed by crystal violet assay. Metabolites in CFS were identified using GC-mass spectrometry-based analysis.

#### RESULTS AND DISCUSSION

The results showed that sub-MIC concentrations of cell-free supernatants of *L. plantarum* and *L. reuteri* significantly inhibited biofilm formation. The highest percentage of destruction of 24 h-old biofilms after treatment with 5MBC dose were obtained. The results were confirmed by scanning electron microscopy (SEM). It should be noted that supernatants neutralized with NaOH did not show any antibacterial activity.

#### CONCLUSION

According to the results obtained in this research, CFS of *L. plantarum* and *L. reuteri* have promising antimicrobial and anti-biofilm activity to inhibit and destroy the biofilm of pathogenic strains.

**Keywords:** Biofilm; Cell Free Supernatant (CFS); Antibiofilm; *L. Plantarum*; *L. Reuteri*; *P. Aeruginosa*; *S. Aureus*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Role of microbial Infections in male infertility

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Multiple factors such as lifestyle, varicocele, hypogonadism, genetic defects, and urogenital tract infections (UTI) are important causes of male infertility. An imbalance in urinary microbiota may cause an overgrowth of pathogenic bacteria and damage various parts of the genital tract, leading to impaired spermatogenesis and male infertility. The treatment of urogenital tract infections are crucial. Previous studies have shown that probiotics can be applied for the treatment of male infertility problems. In this review discusses current research investigating the effects of some bacteria such as *Escherichia coli* (*E. coli*), *Bacteroides ureolyticus* (*B. ureolyticus*), *Chlamydia trachomatis* (*C. trachomatis*) on urogenital tract and sperm parameters.

#### MATERIALS AND METHODS

To collect data from various sources, we conducted an electronic search of the literature using PubMed, Web of Science, Scopus, and Google Scholar.

#### RESULTS AND DISCUSSION

*E. coli*, a gram-negative bacterium, can inhabit the male genital tract and has been associated with infections of the male accessory glands. This bacterium can cause necrotic changes in the Seroli cells and initiate cell death pathways in the seminiferous tubules, which can result in impaired spermatogenesis. *B. ureolyticus* is a gram-negative microorganism that can penetrate the male reproductive system. This is associated with a significantly higher prevalence of three distinct semen abnormalities: (1) reduction in total fructose, (2) increase in short-tailed spermatozoa, and (3) increase in epithelial cell count. *C. trachomatis* has been identified in the male genitourinary tract. *C. trachomatis* could harm sperm indirectly by producing inflammatory mediators like cytokines and reactive oxygen species (ROS). Furthermore, it has been demonstrated that *C. trachomatis* infection can cause anti-sperm antibody (ASA) production in both male and female patients.

#### CONCLUSION

Various bacterial species can colonize distinct areas of the male genital tract, resulting in adverse effects on spermatogenesis and a decline in the quality of sperm.

**Keywords:** Urogenital tract infection, Male infertility, Sperm parameters

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Introducing the potential of a newly isolated yeast strain, SlgEBL5 in untreated polyethylene terephthalate microplastic biodegradation

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Polyethylene terephthalate (PET), a heteroatomic, semi-aromatic polyester, is among the most manufactured and used plastic polymers, only 40% of its post-consuming products are recycled and the remaining are released to nature. Facing these plastic residues to the abiotic factors of the environment will fragment them into micro-sized secondary particles, called microplastics that could enter the food web and reach the human body through biomagnification. Among the methods for microplastic remediation, biodegradation is known as an eco-friendly and cost-effective method to remove environmental pollutants and draw scientific society's attention to removing microplastics in recent years. This study aims to introduce a new PET-degrading yeast strain and assess its biodegradation efficiency.

### MATERIALS AND METHODS

A PET-degrading yeast strain was isolated from activated sludge in the mineral-based carbon-free medium containing PET microplastics as the sole carbon source and selected based on the qualified lipase and esterase assessments. The selected isolate was identified through ITS sequencing and assessed for PET microplastic biodegradation ability through Scanning Electron Microscopy (SEM), Gas Chromatography-Mass Spectroscopy (GC-MS), Fourier Transform Infrared spectroscopy (FTIR), Detection-Light Scattering (DLS), zeta potential analysis, and microplastic's weight loss measurement.

### RESULTS AND DISCUSSION

The results showed that the isolate is a new strain of *Vanrija* sp., that could decrease 10% of the PET microplastic weight after 30 days. SEM micrographs revealed its colonization and crack creation on PET microplastic surface, making them 40.6 times smaller, as the DLS results revealed, and shifting their zeta potential from -19.3 to +31.0. Comparing the FTIR spectrum of treated and untreated microplastics also proved the decrement in PET crystallinity and intensity of the amorphous fragments of this polymer, releasing benzene and alkane derivatives as by-products.

### CONCLUSION

Combining these findings, it can be concluded that *Vanrija* sp. SlgEBL5 has an acceptable degrading function as a biological treating agent for PET microplastics.

**Keywords:** Biodegradation, Microplastic, Polyethylene Terephthalate, *Vanrija* sp.

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# Investigating the prevalence of CRISPR-Cas systems and their association with antibiotic resistance in *Enterococcus faecalis* and *Enterococcus faecium* isolated from hospitalized patients

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Enterococcus faecalis* and *Enterococcus faecium*, are an important cause of nosocomial infections. Antibiotic resistance of *Enterococci* is a challenge in the clinical setting and increases the difficulty of treating *Enterococcal* infectious diseases.

Clustered regularly interspaced short palindromic repeats (CRISPR) and their CRISPR-associated proteins (Cas) are an adaptive immune system involved in specific defenses against the invasion of foreign elements. Three Type II CRISPR systems (CRISPR1-*cas*, CRISPR2 and CRISPR3-*cas*) have been identified in *Enterococci* isolates.

This study aimed to evaluate the presence of CRISPR-Cas system genes and their possible association with antibiotic resistance patterns of *E. faecalis* and *E. faecium* species isolated from hospitalized patients.

### MATERIALS AND METHODS

In this study, a total of 62 isolates of *E. faecalis* and *E. faecium* were collected from urinary tract infections (UTI), Blood infections, Wound infections and other sources. The isolation and identification of *Enterococci* species were performed by standard bacteriology tests and polymerase chain reaction (PCR). Antibiotic susceptibility profiles were determined by the disc diffusion method and studied using standard CLSI protocols.

The presence of various CRISPR-Cas systems was investigated by PCR. The association of the occurrence of CRISPR-Cas systems with antibiotic resistance was analyzed with appropriate statistical tests.

### RESULTS AND DISCUSSION

The results of PCR confirmed the prevalence of 32 (51.6%) *E. faecalis* and 30 (48.3%) *E. faecium*, respectively.

In total, 53 (85.4%) of 62 isolates showed the presence of CRISPR-Cas loci. The incidence of CRISPR-Cas was more common in *E. faecalis*.

CRISPR1, CRISPR2, and CRISPR3 were present in 15 (24.1%), 52 (83.8%), and 12 (19.3%) *Enterococci* isolates, respectively. The CRISPR-Cas positive isolates showed significant lower resistance rates against Linzolid and Chloramphenicol in comparison with CRISPR-Cas negative isolates. The results showed that the presence of CRISPR-Cas genes was lower in multidrug-resistant (MDR) isolates (77.1%, n=27/35) compared to the non-MDR enterococci isolates (96.2%, n = 26/27).

### CONCLUSION

According to our study, the lack of CRISPR-Cas genes was associated with more antibiotic resistance rates and multidrug resistance in *E. faecalis* and *E. faecium* isolated from clinical samples.

**Keywords:** *Enterococcus faecalis* – *Enterococcus faecium* – CRISPR-Cas system – Antibiotic resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Phage therapy: Opportunities & Challenges

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#### ABSTRACT

Emerging antibiotic resistant bacteria as a severe health care concern, threatens the humans worldwide. Developing new effective alternatives to antibiotics is critical in therapeutic approaches. Bacteriophages as natural antibacterial agents can play significant role to control antibiotic-resistant bacterial infections. This assay provides an overview of phage therapy today, its outcome in the future and the way of using unique characteristics and potential of phages in phage therapy. Also, it leads to address the challenging barriers preventing of clinical settings to use the phages in therapeutic purposes. In spite of clinical potential of phages, some challenges in the field of biology, safety, economy and... should be investigated to further implementation and acceptance of phages in treatment of bacterial infections.

**Keywords:** bacteriophage, infection, antibiotic resistance, phage therapy

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Exploring the Potential of *Bacteroides Thetaiotaomicron* in Modulating the Immune Response in Acute Myeloid Leukemia

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Bacteroides thetaiotaomicron* is proposed as a potential candidate for the next generation of probiotics. The ES-8 and ES-D genes play crucial roles in activating and modulating the innate immune system against leukemia following bacterial exposure in the bone marrow. This study was designed to examine the effect of *B. thetaiotaomicron* and its derivatives on alterations in the expression of ES-8 and ES-D genes, and their significance in modulating the severity of acute myeloid leukemia (AML).

### MATERIALS AND METHOD

The impact of *B. thetaiotaomicron*, outer membrane vesicles (OMVs), inactivated bacteria, and supernatant treatments on the ES-8 and ES-D gene expression in the KG-1 cell line was analyzed using the quantitative reverse transcription-polymerase chain reaction (qRT-PCR) method. The Livak ( $\Delta\Delta CT$ ) method was employed to interpret the qRT-PCR results.

The extraction and evaluation of outer membrane vesicles from gram-negative bacteria were performed using ultracentrifugation and ultrafiltration-based methods.

### RESULTS AND DISCUSSION

The KG-1 cell line showed a significant response to treatment with live and active *B. thetaiotaomicron*, particularly in ES-8 and ES-D transcription. OMVs from this bacterium, at a concentration of 50  $\mu\text{g/ml}$ , significantly intensified the expression of the ES-8 ( $p=0.01$ ) and ES-D ( $p=0.02$ ) genes. This effect was even more pronounced at a concentration of 100  $\mu\text{g/ml}$ . Inactivation at MOI 10 ( $p=0.03$ ) and MOI 50 ( $p=0.003$ ) significantly induced transcription of both genes. Additionally, a 25% supernatant considerably augmented the transcriptional expression of ES-8 ( $p=0.038$ ) and ES-D ( $p=0.034$ ) genes.

### CONCLUSION

Our findings suggest that OMVs at a concentration of 100  $\mu\text{g/ml}$ , inactivated bacteria, and supernatants of *B. thetaiotaomicron* play a crucial role in shaping the immune response and could be considered as potential postbiotic and paraprobiotic candidates for further research. Moreover, our data indicate a significant reduction in the severity of acute myeloid leukemia in the erythroleukemia phase, affirming its potential as an effective adjuvant treatment for leukemia.

**Keywords:** *B.thetaiotaomicron*, Acute myeloid leukemia, Microbiota, OMVs, ES-8, ES-D

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluating the Sporicidal Activity of a sterilizing agent against *Bacillus subtilis* spores

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Today, hospital infections are one of the main health problems worldwide, especially in developing countries. Meanwhile, sterilization plays a very important role in reducing the risk of hospital infections. This study was conducted to determine the sporicidal effect of Steril- C based on peracetic acid on medical surfaces and instruments.

### MATERIALS AND METHODS

This study was conducted based on the dilution-neutralization test according to the DIN EN 14347 protocol. In this test, after exposing the suspension containing spores of *Bacillus subtilis* to the disinfectant during the selected times and neutralizing the effect of the disinfectant, a culture was done on a blood agar medium, and after 24 hours, the number of colonies was counted.

### RESULTS AND DISCUSSION

Steril- C disinfectant solution was able to reduce 6 log number of microorganisms within 5 minutes in the vicinity of *Bacillus subtilis* spore suspension, and after 5, 10, 20, 30, 60, and 120 minutes, no colonies on Blood agar culture medium were observed. The main purpose of infection control is to prevent the spread of microorganisms or pathogens. The study found that Steril-C has strong and rapid sporicidal effects, reducing the number of spores by 6 logs within 5 minutes in the test suspension. . Previous laboratory studies have also shown that Peracetic acid can inactivate bacteria in under 5 minutes at concentrations less than 100 ppm, Therefore, Peracetic acid is a broad-spectrum and high-level disinfectant that can rapidly kill viruses, bacteria, fungi, and bacterial spores effectively, with the additional benefit of environmental protection.

### CONCLUSION

Steril- C disinfectant solution can destroy all the spores in the suspension within 5 minutes, so this material can be used as a high-level disinfectant with fast and strong effectiveness for the sterilization of medical equipment in hospitals.

**Keywords:** Disinfectant, *Bacillus subtilis*, Spores, Peracetic acid

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Improving the thermal stability of Cel5E of *Clostridium thermocellum* by protein engineering of flexible regions

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#### ABSTRACT

**BACKGROUND AND ABJECTIVE:** Site directed mutagenesis in protein flexible regions is an efficient protein engineering method to increase thermal stability. In order to have wider industrial applications of enzymes such as Cel5E of *Clostridium thermocellum*, high thermal stability is necessary.

**MATERIALS AND METHODS:** Modeling of native CEL5E and mutant enzyme was done based on homology modeling using swissmodel server. The evaluation of the predicted models was done based on GMQE and QMEAN4 parameters and also in the SAVES server. Flexible regions in CEL5E were predicted using molecular dynamic simulation methods and GROMACS. The binding affinity of native and mutant enzyme to cellulose substrate was evaluated by AUTODOCK VINA. After introducing the flexible regions, the mutagenesis candidates were predicted by SDM, CUPSAT, POP MUSIC, HOT MUSIC and FIREPROT servers.

**RESULTS AND DISCUSSION:** The results showed that in CEL5E enzyme, the residues in position 58-66 have the most flexibility and the highest RMSF (root mean square fluctuation) value. In this region, R63H mutation was predicted and introduced as the best enzyme stabilizing mutation based on  $\Delta\Delta G$  and  $\Delta T_m$ .

**CONCLUSION:** The results of this research led to the introduction of hotspots, th introduction of mutations producing commercial enzyme strains and enzyme isoforms with the best stability. By performing advanced computational tests in quasi-real conditions, it was possible to obtain the best novel isoforms that are more capable than the existing native isoforms.

**Keywords:** Thermal stability, CEL5E, Flexible region, Site directed mutagenesis.

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# Evaluation of antibiotic susceptibility, prevalence of extended-spectrum beta-lactamase (ESBL), antimicrobial resistance (MDR), and biofilm formation ability in clinically isolated *Escherichia coli*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Escherichia coli* (*E. coli*) is an opportunistic bacterium with high prevalence that has high antimicrobial resistance (AMR). Investigating the sensitivity pattern, biofilm formation ability and presence of colistin-resistant *mcr-1*, *2* genes in multi-drug resistant (MDR) *E. coli* bacteria is one of the main goals of this study. After biochemical and molecular confirmation tests, sensitivity test, biofilm formation and minimum inhibitory concentration to colistin were performed on 100 *E. coli* isolates.

### MATERIALS AND METHODS

Sample collection and identification of 100 bacterial isolates was done. Antibiotic susceptibility testing was performed based on disc diffusion method. ESBL phenotypes were detected through the results of the Antibiogram. Semi- quantitative biofilm formation assay was done. MIC for colistin antibiotic was measured using microtiter plate assay. Total DNA was extracted. Molecular detection of the target genes was performed. Statistical analysis was done.

### RESULTS AND DISCUSSION

Disc diffusion method showed that the highest sensitivity was against imipenem (98%), amikacin (96%) and gentamicin (81%), respectively. Among the isolated *E. coli*, 47 strains (47%) were determined as extreme beta-lactamase (ESBL) phenotype that respectively, 45%, 64%, 85% of them are able to forming weak, moderate and strong biofilm, and 53% of they were unable to produce biofilms. 72 (72%) were determined as MDR, 59 strains (94.81%) of MIC>2 are resistant and 1 strain (100%) of MIC≤2 are sensitive to colistin. *mcr-1* gene was found in 26 (36.11%) colistin-resistant strains and 1 (1.38%) colistin-sensitive isolate. 37.49% of MDR positives have *mcr-1* and no *mcr-2* gene was detected in the isolates. Half of the *mcr-1* are able to produce biofilm.

### CONCLUSION

A high level of resistance was observed in ampicillin, trimethoprim sulfamethoxazole and colistin which can be prevented with community awareness and appropriate health policies.

**Keywords:** *Escherichia coli*, Antibiotic resistant, Colistin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Designing anticancer peptides based on a bacteriocin of *Streptococcus gallolyticus* using in silico methods

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Cancer is one of the life-threatening diseases in which biological factors, such as bacteria are involved in its development. In recent years, antimicrobial peptides have received much attention for treatment of cancer or microbial disease. Therefore, identification of engineered peptides that can effectively suppress cancer markers, such as epidermal growth factor receptor (EGFR), is a suitable model for introducing anticancer drugs. This study aims to use the amino acid sequence related to a bacteriocin of *Streptococcus gallolyticus*, Gallocin, to design newly engineered peptide(s) with an anticancer activity using in silico or bioinformatics methods.

#### MATERIALS AND METHODS

The anti-cancer sequences with a length of 10 amino acids were predicted from the amino acid sequence of Gallocin with the help of support vector machine (SVM) algorithm web-based tools. Then, four sequences with a high SVM score and the best physiochemical properties, including positive net charge and amphipathicity, were selected for further analysis. The docking studies of receptor-ligand interactions were carried out by Molegro Virtual Docker software and also ClusPro and HDOCK servers. Finally, the pharmacological properties of predicted peptides, including absorption, distribution, metabolism, excretion, and toxicity (ADMET), were evaluated using ADMETlab web tool.

#### RESULTS AND DISCUSSION

Based on Docking results, all peptides showed approximately similar affinity to the EGFR receptor. However, Peptide 4 had the lowest energy (-816.9) and the highest binding affinity to the EGFR receptor. In addition, Peptide 1, Peptide 3, and Peptide 4 formed many reasonable Hydrogen bond interactions and consequently a strong attachment to the EGFR receptor. Also, according to ADMET features, these peptides showed reasonable toxicity and pharmacological properties.

#### CONCLUSION

Overall, some peptides were designed by time and cost-effective in silico methods which can be candidates for further experimental anti-cancer research.

**Keywords:** Molecular docking, Peptide, EGFR, ADMET profiling

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Designing a Stable Fusion Protein Antigen for enhancing ELISA assay sensitivity for SARS-CoV-2 Detection: Molecular Dynamics Simulations and Bioinformatic Studies

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Accurate and sensitive detection of SARS-CoV-2 is crucial for effective control and management of the COVID-19 pandemic. This study employed bioinformatic approaches, including molecular dynamics (MD) simulations, to design a stable and efficient antigen for SARS-CoV-2 detection. The fusion protein, CoV2-Pro, containing multiple domains from the Omicron and Delta variants, including Receptor Binding Domain (RBD) and the nucleoprotein, was investigated for its structural stability and antigenic properties.

### MATERIALS AND METHODS

CoV2-Pro was designed using in-silico cloning and 3D modeling, employing the PHYRE2 Protein Fold Recognition Server. Subsequently, physicochemical properties were analyzed to evaluate the protein's stability and antigenicity. To gain insights into the structural dynamics and stability of CoV2-Pro, molecular dynamics (MD) simulations were conducted, spanning 100 nanoseconds. Moreover, for evaluation of the binding affinity between CoV2-Pro and two SARS-CoV-2 human IgG1 neutralizing monoclonal antibodies (mAbs), namely Bebtelovimab LY-CoV1404 (PDB: 7MMO) and LY-CoV488 (PDB: 7KMH), was assessed through molecular docking simulations.

### RESULTS AND DISCUSSION

Bioinformatic studies indicated that CoV2-Pro adopts a stable 3D conformation with suitable antigenic characteristics. MD simulations revealed the protein's structural stability and conformational changes during the simulation period. Molecular docking simulations demonstrated strong binding interactions between CoV2-Pro and SARS-CoV-2 antibodies, highlighting its potential as an effective diagnostic antigen.

### CONCLUSION

Using bioinformatic techniques and MD simulations, we successfully designed a stable fusion protein, CoV2-Pro. This protein holds great promise as a robust antigen for SARS-CoV-2 detection. Our study provides valuable insights for developing advanced diagnostic strategies to accurately identify SARS-CoV-2 infections, contributing to the global effort to combat the COVID-19 pandemic.

**Keywords:** SARS-CoV-2 diagnosis, Fusion proteins, RBD Domain, Nucleoprotein Domain, Molecular dynamic, COVID-19

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# The frequency of *vanB* and *vanA* genes in Vancomycin Resistant *Staphylococcus aureus* isolates obtained from dialysis patients

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### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Hemodialysis is the most common method of alternative treatment in kidney patients. In these patients, due to weak immune system and continuous use of venous catheter for dialysis, the possibility of hospital infection is high. The most common cause of infection in these patients is *Staphylococcus aureus* bacteria. Vancomycin is now identified as a last resort for the infection control. The aim of this study is to investigate the prevalence of resistance to methicillin and vancomycin in *Staphylococcus (S.) aureus* isolates collected from dialysis patients of Imam Reza Hospital in Tabriz, Iran

#### MATERIALS AND METHODS

Swab Samples were obtained from nasal, throat and catheter of 230 dialysis patient at Imam Reza Hospital in Tabriz, Iran. the isolation of *S. aureus* bacteria was performed by conventional microbiological methods. Antibiotic sensitivity patterns of isolates were determined by disk diffusion method and E-test. The frequency of *Van A*, *Van B* and *mecA* genes was detected by PCR reaction.

#### RESULTS AND DISCUSSION

Among the 170 *S. aureus* isolates collected from hemodialysis patients in this investigation, the highest resistance was observed to Ampicillin (79.41%), while the highest sensitivity was observed against Rifampin (82.94%). For the detection of vancomycin resistant isolates, E- test was performed for 35 isolates that was found as vancomycin resistant isolates based on disk diffusion results. The results showed that 37.14% of the samples were resistant, 14.28% were sensitive, and 20% showed intermediate resistance to vancomycin. According to the PCR results, 29.41% of patients were positive for *mecA* gene, 5.29% for the *vanA* gene, and 7.05% were positive for the *vanB* gene. All the isolates carrying *vanA* and *vanB* were resistant to Ampicillin (7.05%), Cefoxitin (5.29%), Erythromycin (4.70%), Methicillin (3.52%), and Oxacillin (3.52%). The number of female patients carrying the vancomycin-resistant gene was higher than the number of men ( $p < 0.05$ ). Based on results, there is a significant relationship between diabetes and high blood pressure prevalence and resistance to methicillin and vancomycin.

#### Conclusion

Because of the increasing prevalence of resistance to methicillin and vancomycin, it is necessary to propose an appropriate antibiotic pattern to halter resistant infections in patients with weak immune system like hemodialysis patients.

**Keywords:** Dialysis Patients, *S. aureus*, Vancomycin Resistance, Methicillin Resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Mutation in *mgrB* is the major colistin resistance mechanism in *Klebsiella pneumoniae* clinical isolates in Tehran, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Colistin is considered as one of a last resort antimicrobial agent against multidrug-resistant Gram-negative bacteria including *Escherichia coli* and *Klebsiella pneumoniae*. However, the recent emergence of colistin resistance (ColR) worldwide that severely restricts therapeutic options is a serious threat to global public health. In this study we have investigated the molecular determinants in ColR *K. pneumoniae* isolates collected from clinical specimens.

### MATERIALS AND METHODS

A total of 98 *E. coli* and 195 *K. pneumoniae* clinical isolates were collected from two hospitals from August 2018 to December 2019 in Tehran, Iran. Colistin susceptibility and minimum inhibitory concentrations (MIC) were determined according to the Clinical and Laboratory Standards Institute by disk diffusion method, and microdilution method, respectively. For isolates with colistin MIC  $\geq 4$  mg mL<sup>-1</sup>, PCR was performed for the detection of *mcr-1* to *mcr-4* genes. Moreover, nucleotide sequences of *mgrB*, *phoP*, *phoQ*, *pmrA*, and *pmrB* genes were determined by sequencing. Finally, the transcriptional level of *pmrK* and *pmrC* genes was evaluated by quantitative reverse transcription PCR (RT-qPCR).

### RESULTS AND DISCUSSION

None of the *E. coli* isolates were resistant to colistin while 21 out 195 *K. pneumoniae* isolates were identified as resistant, 19 of which carried mutation in the *mgrB* gene. Three different mutations were observed in the *pmrB* gene in 3 *K. pneumoniae* isolates. None of the ColR isolates showed alternations in *pmrA*, *phoP*, and *phoQ* genes. Furthermore, none of the plasmid-encoding genes were detected. Transcriptional level of the *pmrK* gene increased in all ColR isolates meanwhile, *pmrC* overexpression was detected in 16 out 21 (76.19%) isolates. Eventually, all ColR isolates were susceptible to tigecycline.

### CONCLUSION

Our results demonstrated that the alternation of *mgrB* gene is the main mechanism related to colistin resistance among ColR *K. pneumoniae* isolates in this study.

**Keywords:** colistin resistance, colistin, *Klebsiella pneumoniae*, *mgrB*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Enhancing Cell Viability and Immunogenicity of OmpA as a Promising Subunit Vaccine Candidate Against Cytotoxic Effects of *A. baumannii*

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## ABSTRACT

### BACKGROUND AND AIM

*Acinetobacter baumannii* is a significant opportunistic pathogen and a major concern in healthcare settings due to its involvement in nosocomial infections and high antimicrobial resistance. The alarming mortality rate among infected patients underscores its success as a pathogen. Outer membrane protein A (OmpA) has been identified as a crucial virulence factor associated with the survival and pathogenicity of *A. baumannii*. OmpA interacts with eukaryotic cells, leading to cytotoxicity by binding to death receptors on the cell surface. Additionally, OmpA plays a significant role in bacterial pathogenesis by interacting with epithelial cells, inducing apoptosis, and inhibiting complement activation.

### METHODS

The OmpA was expressed and purified. The purified protein was then injected into groups of mice to induce the production of anti-OmpA antibodies. HeLa cells were plated at 70% confluency. The standard strain of *A. baumannii* ATCC 19606 and a clinical isolate, *A. baumannii* 58ST, were exposed to Anti-OmpA serum. The cells were incubated with the bacteria-cell solution overnight. MTT test was performed.

### RESULTS

OmpA was successfully expressed, purified, and visualized as ~ 38kDa on SDS-PAGE. An increased antibody titer was achieved as measured by indirect ELISA. Cells exposed to the sera showed lower infections than the control group.

### CONCLUSION

The presence of anti-OmpA antibodies enhances cell viability against the cytotoxic effects of *A. baumannii*. OmpA demonstrates immunogenicity and holds promise as a candidate for the development of an effective subunit vaccine against *A. baumannii* infections.

**Keywords:** *Acinetobacter baumannii*, OmpA, Cytotoxic effects, HeLa cell line, Subunit vaccine

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Modulation of Wnt Signaling Pathways by *Lactobacillus acidophilus* Postbiotics Suppresses Proliferation and Migration of HT-29 Colorectal Cancer Cells: A Comprehensive Integrated *In Silico* and *In Vitro* Analysis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Colorectal cancer (CRC) is one of the most prevalent and life-threatening cancers worldwide. Located near the surface of the colorectal epithelium, the gut microbiota comprises a large population of microorganisms that interact with host cells to regulate many physiological processes. However, disruption of the gut microbiota has been confirmed to be related to gastrointestinal diseases such as CRC. Recently, the beneficial role of postbiotics, a new concept in describing microorganism-derived substances in CRC, has been uncovered by various studies. But, a comprehensive characterization of the molecular identity, mechanism of action, or routes of postbiotic administration, notably their role in CRC, is still lacking. Today, recent advances in technology, such as single-cell RNA analysis (scRNA-seq), have enabled us to gain a deeper and more precise understanding of CRC, including the signaling pathways that are affected by biomolecules like postbiotics.

### MATERIALS AND METHODS

This study used scRNA-seq analysis to identify differentially expressed genes (DEGs) between cancerous and normal adjacent tissue. Enrichment analysis determined the main pathways in which these genes were involved. The Real-Time quantitative PCR (RT-qPCR) was carried out to verify the expression of selected genes on CRC and normal adjacent tissues. Furthermore, we studied the effect of postbiotics obtained from *Lactobacillus acidophilus* (*L. acidophilus*) on the proliferation and migration of HT-29 cells using MTT and scratch assays. Finally, we evaluated the effect of *L. acidophilus* postbiotic on the expression of selected genes in the HT-29 cell line.

### RESULTS AND DISCUSSION

Our scRNA-seq analysis revealed the presence of four DEGs named *SFRP1*, *SFRP2*, *SFRP4*, and *MMP7* in carcinoma vs normal adjusted tissues. Subsequently, enrichment analysis determined that these DEGs are involved in the Wnt signaling pathway as a primary cascade

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in CRC. RT-qPCR experiments on tissues confirmed that in the carcinoma stage, the expression of *SFRP1*, *SFRP2*, and *SFRP4* was decreased, while the expression of *MMP7* was increased. Finally, we revealed that *L. acidophilus* postbiotics had anti-proliferative and anti-migration effects on HT-29 cells, while it did not exert anti-proliferation activity on control fibroblasts. We also demonstrated that treating HT-29 cells with postbiotics can increase the expression of *SFRP1* and *SFRP2* whereas decreasing the *MMP7* expression. Accordingly, no significant changes were noticed in *SFRP4* expression in treated cells.

### CONCLUSION

Our study provides evidence that postbiotics extracted from *L. acidophilus* have an anti-proliferation and anti-migration effect on colorectal cancer cell lines, likely through their modulation of key genes in the Wnt signaling pathway. Our findings suggest that postbiotics may have therapeutic potential in treating CRC. Further investigation into the mechanisms underlying these effects and the optimization of postbiotic formulation and dosage is warranted to advance their clinical application.

**Keywords:** Colorectal Cancer, Postbiotics, *Lactobacillus acidophilus*, Single-cell RNA sequencing, Wnt signaling pathway

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# Bread baking: Using a high throughput Online CO<sub>2</sub> production monitoring method to screening *Saccharomyces cerevisiae* strains for industrial purpose

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Saccharomyces cerevisiae* is a commonly used microorganism in producing of bread and bioethanol. Fermentation is a crucial step in its production process, during which the sugar substrate is converted into carbon dioxide and ethanol. This study compared different microorganisms' CO<sub>2</sub> production levels using two devices that our research team developed. The goal was to determine which microorganisms would be best for bread baking.

### MATERIALS AND METHODS

One hundred strains of *Saccharomyces cerevisiae* from Khorasan Razavi, Iran were screened using a high-throughput pH-based system to identify CO<sub>2</sub> producers. The screening was done using a 96-well plate format, where a 3D-printed silicone lid captured CO<sub>2</sub> emissions from the fermentation well and transferred them to a pH indicator reagent. The strains that caused significant color changes underwent a quantity test using a CO<sub>2</sub> flow meter. The best-performing strain was compared to a commercial strain for dough rising.

### RESULTS AND DISCUSSION

The result of primary screening with various yeast strains led to different colors, from dark blue to green, based on the amount of carbonic acid formed. The flow meter revealed a fermentation profile of promising strains. The highest peak of CO<sub>2</sub> production among strains occurred at different times. The isolate produced more CO<sub>2</sub> than the commercial strain for dough rising.

### CONCLUSION

Our results suggested that screening wild-type *Saccharomyces cerevisiae* with high throughput online CO<sub>2</sub> production monitoring method can lead to finding new strains for dough rising and other industrial purposes.

**Keywords:** *Saccharomyces cerevisiae*, Dough rising, Online monitoring, CO<sub>2</sub> monitoring

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# New modified diagnosis method for detection Demodex mites in affected people with demodicosis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Demodex mites are the most common human ectoparasites. Demodex species can be found on all skin types across a broad geographical range. Demodex mites reside in the pilosebaceous units of the skin. Demodex mites feed on epithelial and glandular cells as well as sebum typically secreted by active pilosebaceous units. Demodicosis is the infestation of Demodex mites on the face, whereby a minimum of 5 mites/cm<sup>2</sup> exist and induce symptoms such as redness of the skin (erythema), telangiectasia, itching, heat, scaling, papules, pustules, and dermatitis, usually accompanied by a burning or pruritic sensation. Bacterial folliculitis, rosacea, seborrheic dermatitis and other common skin conditions have been linked to infestation by Demodex mites (human demodicosis). The diagnosis of demodicosis should be established by the visualization of Demodex mites in high numbers.

### MATERIALS AND METHODS

We used new method for observation Demodex mites precisely. Considering the diameter of the head of sampling tool is 2 mm, in order to sample an area of 1 square centimeter of the skin, it is necessary to sample the sebum of 5 areas of facial skin with a length of 10 mm. These five areas include 10 mm areas from the top of both eyebrows, 10 mm areas from the right and left side of the nose, and a 10 mm area from the border line of the forehead and five areas of different place on scalp. Finally, we have collected a diameter of 1 square centimeter or 100 square millimeters. After transfer of sebum samples to drop of oil on a laboratory slides, a drop for facial skin and scalp, separately; Then we put a cover slide on each drop. The preparation was examined under a light microscope (Olympus SZX16 microscope) 40× and 100× magnification. Multiple sections (about 5 on average) on every slide were examined. All forms of Demodex follicularum and Demodex brevis mites: adult, larvae and egg forms were counted as results. (A positive level of  $\geq 5$ /cm<sup>2</sup> area was considered as a criterion for demodex positivity in patients). The measurements were taken at room temperature (20 °C). exclusion criteria was using sunscreen, foundations and moistures on face that would change the sebum level within the last 2 days.

### RESULTS AND DISCUSSION

In this study we have modified Current diagnostic method and improve the defects of previous methods such as skin surface biopsy (SSB), Dermoscopy and etc. This new modified method has the advantage of being noninvasive, inexpensive, accurate and rapid.

### CONCLUSION

We investigated a new sampling method for determining the presence mites and the severity of Demodex infestation, in both the patient and control groups. Methods commonly used to determine demodex mite densities have many defects such as being time consuming and require specific equipment and a trained observer. The results of our study revealed that our new method based on direct microscopic identification of Demodex mites is a more sensitive method for detecting Demodex mite than SSSB and Dermoscopy. Our sampling method used was not invasive and results are more precise, fast and inexpensive.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating the antibiotic resistance pattern of *Streptococcus iniae* and *Yersinia ruckeri* isolated from rainbow trout in North Khorasan province, Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVE

During the last few years, streptococcosis and yersiniosis have been important problems in cold water fish farms in Iran, causing huge economic losses to the rainbow trout production industry. Knowing the level of resistance and sensitivity of problematic bacteria such as *Yersinia ruckeri* and *Streptococcus iniae* in rainbow trout breeding farms plays an effective role in the appropriate and correct selection of antibiotics and infection control in breeding ponds.

#### MATERIALS AND METHODS

In this research, considering the prevalence of streptococcosis and yersiniosis, 100 pieces of rainbow trout with clinical symptoms of streptococcosis and yersiniosis were harvested from rainbow trout farms located in North Khorasan province in the hot seasons. After performing bacteriological culture and isolation of *Streptococcus iniae* and *Yersinia ruckeri* species, it was finally confirmed by using PCR test with specific primers. Antibiotic sensitivity of bacteria was investigated by disc diffusion method by culturing on Mueller Hinton agar culture medium.

#### RESULTS AND DISCUSSION

The results of bacteriological and molecular diagnosis show that the main cause of bacterial diseases in rainbow trout in North Khorasan province is *Yersinia ruckeri* (87%). And in 13% of disease cases, *Streptococcus iniae* was also detected. The results of the antibiogram of *Yersinia ruckeri* isolates showed that they have the highest resistance to Amoxicillin, Tylosin, Erythromycin, Gentamicin and the highest sensitivity to Enrofloxacin, Florfenicol, Lincospectin, and Oxytetracycline. *Streptococcus iniae* isolates also showed the highest sensitivity to Enrofloxacin, Florfenicol, Doxycycline, and the highest resistance to Bacitracin, Penicillin, and Trimethoprim, respectively.

#### CONCLUSION

In order to prevent the increase of resistance, the indiscriminate use of antibiotics should be prevented and bacterial culture and antibiogram test should be done before use. Self-medication and indiscriminate use of antibiotics will cause drug resistance in pathogenic bacteria and irreparable consequences such as drug residue and drug resistance in humans.

**Keywords:** *Yersinia ruckeri*, *Streptococcus iniae*, Antibiotic resistance pattern

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Genetically derived toxoids; a new approach to produce clostridial toxoid vaccines

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The current clostridial vaccines are composed of chemically detoxified toxins (toxoids). Today with molecular biology and biotechnology advances, low-toxic or non-toxic derivatives of clostridial toxins can be obtained by site directed mutagenesis of the toxin genes. The aim of this study was to design and produce the genetically derived toxoids of *Clostridium perfringens* toxins.

### MATERIALS AND METHODS

Various mutants were created in specific locations of *C. perfringens* toxin genes, using mutated complementary primers based on the overlap-extension PCR technique. These specific genes were cloned in *Escherichia coli* DE3 strain and toxin mutants devoid of any toxic activity has been produced and evaluated with in vivo and in vitro studies.

### RESULTS AND DISCUSSION

The results showed that genetically derived toxoids of *C. perfringens* toxins could be well expressed in *Escherichia coli*. These toxoids were safe in in vivo and in vitro evaluations and were able to stimulate mucosal and parenteral immune responses.

### CONCLUSION

These genetically derived toxoids of *C. perfringens* toxins are superior to the classical toxoids both in safety and in immunogenicity. Thus they should replace the old toxoids in the existing clostridial vaccines and can be used to produce a new generation of toxoid vaccines.

**Keywords:** *Clostridium perfringens*; Genetically derived toxoids; Toxin; Vaccine

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Lichens and their impacts on cultural heritage: current methods and future strategies for their removal

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Lichens as one of the important biodeteriorating organisms have a main role in the biodeterioration of cultural heritage monuments, although in some conditions, they show protective impacts and have a conservative effect to protect substrate damages. Since lichens mainly have deleterious effects, it is necessary to remove or mitigate with proper methods.

Lichens with fungal hyphae penetration can able to cause to physical damage and with material secretion by mycobionts and photobionts can lead to chemical damage. Lichen produce encrustations in the stone material and can be cause biodeterioration in cultural heritage. Oxalic acid secreted by lichens mycobiont leads to chemical damage and chelate metal ions such as calcium and therefore widespread erosion in their substrata. The aim of this study was to overview on dual role of lichens in cultural heritage and strategies for control and remove of destructive lichen.

### MATERIALS AND METHODS

Articles related to the subject were searched in Scopus, Science Direct and Web of Science databases and articles that evaluated the effects of lichens in biodeterioration and bio-protective of cultural heritage substrate and strategies for their mitigation were included in the study.

### RESULTS AND DISCUSSION

According to studies, in cases where lichens have protective effects or have slight deteriorative effects, create aesthetic in the substrate or are of biodiversity value, the removal of lichens is questionable. But in general, in the protection of cultural heritage, the connection between lichen and the deterioration of the building is always investigated and the removal of lichen in restoration is usually considered.

Currently, different cleaning methods are used to eliminate lichens' colonies and thus reduce their negative effects. These methods were categorized in three groups. Mechanical methods including brushing with metal tips and the scalpel, chemical methods using biocides and physical methods using laser ray irradiation are three main approaches to clean the monuments. Today, natural biocides such as essential oil are used in many indoor sites to control the biodeteriorant agents, which are friendly environment approach. The combination of this with other methods can increase the efficiency of those methods and reduce the dosage of the chemicals and irradiation.

### CONCLUSION

Lichens have innately deleterious effect on stone integrity. Deterioration mechanism by lichens is mostly due to adhesion, penetration and volume changes of hyphal structures and the release of acidic or chelating metabolites. Lichens via protection and installation facilitation of degradative microorganisms can play role in the biodeterioration of the substrate.

Although there are different methods for remove or mitigation in cultural heritage in order to avoid undesirable effects to some of them it is necessary to investigate on improving technology or using the combination of cleaning methods to reach a more favorable effect.

**Keywords:** Lichen, Biodeterioration, Protective, Cultural heritage, Conservation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Antifungal susceptibility pattern of clinical isolates of *Candida parapsilosis*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Candida* species is one of the most common types of fungal infection in the world, which can opportunistically cause disease in people if there is a defect in the immune system or mucosal defense. *Candida parapsilosis* is a major human pathogen whose prevalence has grown significantly in the last two decades. Considering the increasing resistance of this species to antifungals, this study was conducted with the aim of determining the pattern of drug resistance in order to more effectively treat the infections caused by *C. parapsilosis*.

#### MATERIALS AND METHODS

*Candida* isolates were all cultured on Sabouraud Dextrose Agar medium for 48 h at 28 °C and then sub-cultured on a chromogenic medium. Final identification was confirmed by PCR RFLP method. Antifungal susceptibility experiment was assessed using CLSI (Clinical and Laboratory Standard Institute) guidelines for determination of MIC (Minimum inhibitory concentration) of three antifungal drugs including fluconazole, voriconazole and itraconazole.

#### RESULTS AND DISCUSSION

According to the antifungal susceptibility test results, the MIC ranges for fluconazole, voriconazole and itraconazole were 0.25-4, 0.0313-0.25 and 2-16 µg/ml, respectively. The analysis of MICs showed that most isolates of *C. parapsilosis* were sensitive to fluconazole and itraconazole but most of the isolates were resistant to itraconazole.

#### CONCLUSION

According to our results, the best antifungal that can be chosen for *C. parapsilosis* is fluconazole and voriconazole. Itraconazole may not be suitable for the treatment of *C. parapsilosis*.

**Keywords:** *Candida parapsilosis*, Clinical isolates, Drug resistance, MIC

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# A newly designed label-free immunosensor with reduced Graphene Oxide & Au Nanoparticles on glassy carbon electrode for *Escherichia coli* detection in real samples

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Escherichia coli* is an indicator in the quality control of pharmaceutical and other samples. Current methods for *E. coli* detection are time consuming and expensive. Biosensr is one of instruments for bacterial rapid detection.

### MATERIALS AND METHODS

In this study, reduced graphene oxide (rGO) as carbon composition was immobilized on glassy carbon electrode (GCE). Chronoamperometric and reduction methods were used to decorate Au NPs and modification was completed with polyclonal *E. coli* antibody and 0.5 W/V% Bovine Serum Albumin solution. SEM was used to verify morphology and structure of rGO and Au NPs as well as the surface of bare glassy carbon electrode before and after modification. *E. coli* in different samples which prepared in 0.1M PBS (pH 7.4) and mixed with 0.5mM acetaminophen was investigated with Square-Wave Voltammetry and Cyclic Voltammetry techniques. In comparison with biosensor, classical detection method was performed with serial dilutions of *E. coli* ATCC 8739 ( $1 \times 10^1$ – $1 \times 10^8$  CFU/ml).

### RESULTS AND DISCUSSION

Although, two methods of Au NPs immobilization were used, SEM pictures established that current deviation with Au was not applicable in GC/rGO/Au NPs/Ab/BSA modification. Currents didn't increase during electrode modification and it wasn't a successful design for *E. coli* detection.

### CONCLUSION

In spite of two methods of Au NPs immobilization, SEM pictures established that current deviation with Au was not applicable in GC/rGO/Au NPs/Ab/BSA modification so it couldn't compare with classic method.

**Keywords:** Reduced graphene oxide, Biosensor, *E. coli*, Au NPs, Immobilization

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Enhancement of the immunogenicity of a *Mycobacterium tuberculosis* fusion protein using ISCOMATRIX and PLUSCOM nano-adjuvants after nasal administration in mice

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Tuberculosis (TB), a contagious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), remains a health problem worldwide and this infection has the highest mortality rate among bacterial infections. Current studies suggest that intranasal administration of new tuberculosis vaccines could enhance the immunogenicity of *M. tuberculosis* antigens. Hence, we aim to evaluate the protective efficacy and immunogenicity of HspX/EsxS fusion protein of *M. tuberculosis* along with ISCOMATRIX and PLUSCOM nano-adjuvants and MPLA through the intranasal administration in mice model.

### MATERIALS AND METHODS

In present study, the recombinant fusion protein was expressed in *Escherichia coli* and purified and used to prepare different nanoparticle formulations in combination with ISCOMATRIX and PLUSCOM nano-adjuvants and MPLA. Mice were intranasally vaccinated with each formulation three times at an interval of 2 weeks. Three weeks after final vaccination, IFN- $\gamma$ , IL-4, IL-17 and TGF- $\beta$  concentration in supernatant of cultured splenocytes of vaccinated mice as well as serum titers of IgG1 and IgG2a and sIgA titers in nasal lavagewere determined.

### RESULTS AND DISCUSSION

According to obtained results, intranasally vaccinated mice with formulations containing ISCOMATRIX and PLUSCOM nano-adjuvants and MPLA could effectively induced IFN- $\gamma$  and sIgA responses. Moreover, both HspX/EsxS/ISCOMATRIX/MPLA and HspX/EsxS/PLUSCOM/MPLA and their BCG booster formulation could strongly stimulate the immune system and enhance the immunogenicity of *M. tuberculosis* antigens.

### CONCLUSION

The results demonstrate the potential of HspX/EsxS-fused protein in combination with ISCOMATRIX, PLUSCOM and MPLA after nasal administration in enhancing immune response against of *M. tuberculosis* antigens. Both nanoparticles were good adjuvants in order to promote immunogenicity of TB fused antigen. So, nasal immunization with these formulations, could induce immune responses and considered as new TB vaccine or as BCG booster.

**Keywords:** *Mycobacterium tuberculosis*, HspX/EsxS, ISCOMATRIX, PLUSCOM, MPLA, Nasal administration.

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# Multifactorial resistance mechanisms associated with tigecycline resistance in *Escherichia coli*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Tigecycline (TGC) is one of the last-resort antimicrobial agents for the treatment of serious infections caused by extensively drug-resistant *Enterobacteriaceae*. TGC resistance is found to be mainly mediated by overexpression of RND-type efflux pumps (AcrAB), mutations in ribosomal S10 protein (*rpsJ*) or plasmid-encoded Tet(A) and acquisition of plasmid-encoded *tetX* family genes.

### MATERIALS AND METHODS

Seven TGC resistant *Escherichia coli* mutants (MICs= 2 to 8 mg/L) were obtained by exposing three different TGC susceptible isolates (MIC= 0.25 mg/L) to increasing concentrations of TGC. Whole genome sequencing was performed to identify genetic alterations associated with reduced susceptibility to TGC. The fitness cost of TGC resistance acquisition was investigated by comparing the *in vitro* growth rate of TGC resistant mutants to that of their wild-type ancestors in an antibiotic-free medium.

### RESULTS AND DISCUSSION

The majority of studied mutants were found to carry genetic alterations in regulators of AcrAB efflux pump. More genetic alterations were also identified in other loci such as those coding for lipopolysaccharide core biosynthesis enzymes as well as ribosomal protein. In most cases but not all, the growth rate of mutants in an antibiotic-free environment was lower than those of wild-type parent isolates indicating presence of fitness cost for TGC resistance acquisition.

### CONCLUSION

The molecular mechanisms involved in TGC resistance among the studied isolates was found to be very diverse, with increased extrusion of antibiotic by efflux pumps being found as the major mechanism.

**Keywords:** Tigecycline resistance, *Escherichia coli*, efflux pump, ribosomal protein

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# Posters



## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Common fungal diseases and related risk factors in Hamadan city

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#### ABSTRACT

Fungal diseases are caused by the growth and reproduction of microscopic fungi inside or on the surface of the human body. Superficial fungal infections are a group of common fungal diseases that involve tissues such as skin, hair, and nails. The purpose of this study was to investigate the frequency and spread of fungal diseases and some factors affecting them in patients. For this purpose, the number of 2549 patients who were referred to the infectious diseases department of Sina Hospital in Hamedan City during the years 2011-2021 were investigated. Data were analyzed with spss-21 software and a chi-square test. Among the 2549 patients who studied, 1089 were women (42.7%) and 1460 were men (57.3%). Among all the patients who studied, the test result of 1757 patients was negative, and 792 patients were positive. The highest frequency of fungal diseases was observed in the age year's group of 11-20 with 519 patients (20.4%). The highest frequency was related to the agent of contact with animals with the number of 620 people (24.3%). Among the positive patients, the highest frequency of fungal diseases was related to mycelium with 511 people (20%); Ectothrix with 90 patients (3.5%), and pseudo mycelium and yeast with 82 patients (3.2%). In terms of gender, 207 women (19%) and 304 men (20.8%) had fungal agents such as mycelium, and 18 women (1.7%) and 72 men (4.9%) had Ectothrix. In the age group of 21-30 years, the most fungal infection with mycelium was observed in the number of 115 people (24.2%), and in the age group of 0-10 years, the highest amount of Ectothrix was observed (53 patients, 11.4%). Among the disease-transmitting agents, the highest rate of fungal infections is related to fungal agents, including mycelium with 200 cases (32.3%), Ectothrix with 41 cases (6.6%), and pseudo mycelium and yeast with 4 cases (0.6 percent) that were transmitted by animals. The results of this study showed that contamination with fungal mycelium and contact with animals was the most important cause of disease infection.

**Keywords:** Fungal Infection, Age, Gender, Hamedan

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Enhancing Antibiotic Efficacy Against *P. mirabilis* Biofilm: The Synergistic Potential of Phage-Antibiotic Combination Therapy

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Proteus mirabilis* bacteria are recognized as a significant etiological agent of urinary catheter infections, especially in cases with abnormalities in urinary tracts. This bacterium can form biofilms on both abiotic surfaces, such as urinary catheters, and biotic surfaces. Bacteriophage therapy emerges as a promising alternative approach for treating infections caused by biofilm-forming bacteria. By complementing conventional treatment methods, bacteriophage therapy enhances treatment specificity and mitigates the adverse effects of certain antibiotics. Additionally, it augments the bactericidal activity against pathogenic bacteria while concurrently safeguarding the natural bacterial flora of the patient's body. Co-administration of antibiotics and phages, known as Phage-Antibiotic Synergy (PAS), represents a strategy to amplify therapeutic efficacy. Phages serve as adjuncts to antibiotics by facilitating a decrease in the antibiotic's minimum inhibitory concentration (MIC) when used in conjunction with phages.

### MATERIALS AND METHODS

First, the clinical isolates of *P. mirabilis* were isolated from the urine of patients with urinary tract infections. The antibiogram test was performed by disc diffusion method according to CLSI 2022 protocol, and Multi-Drug Resistance (MDR) strains were identified. In the next step, the measurement MIC for the antibiotic's cefotaxime and cotrimoxazole were performed by the microdilution broth method (MIC). The Crystal violet microtiter plate assay evaluated the biofilm production of MDR strains. The Checkerboard assay was performed to survey the effect of the combination of Phage and antibiotics. Finally, the effect of phage and antibiotic combination with different concentrations was investigated against the biofilm formation of *P. mirabilis* bacteria, and to determine the living bacteria, colony count and MTT assay were used.

### RESULTS AND DISCUSSION

Based on the findings derived from our study, the simultaneous administration of phages along with cotrimoxazole and cefotaxime antibiotics exhibited a notable reduction of one unit in the minimum inhibitory concentration (MIC) for both antibiotics, as compared to their individual usage. Furthermore, this combined treatment approach demonstrated a significant decline in biofilm production by bacteria isolated from clinical samples ( $P < 0.05$ ).

### CONCLUSION

One of the primary concerns within the healthcare system regarding infections attributed to *P. mirabilis* bacteria is their pronounced propensity for biofilm formation, which poses challenges for individuals utilizing urinary catheters. Moreover, the persistent resistance exhibited by this bacterium towards an assortment of antibiotics in recent years further compounds the issue. Consequently, this alternative therapeutic approach addresses infections induced by biofilm-forming strains of *P. mirabilis* effectively.

**Keywords:** Multi-Drug Resistant, *Proteus mirabilis*, Biofilm, Phage therapy, Phage-antibiotic synergy (PAS)

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Immunological Characterization of Leptospiral Outer Membrane Protein Loa22 in Mice

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Leptospirosis is a worldwide zoonotic disease caused by pathogenic *Leptospira*, which often occurs in tropical and subtropical regions. Focusing on development of rapid diagnostic methods to facilitate early diagnosis and development of a universal vaccine are the main key issues to overcome the disease burden of leptospirosis.

### MATERIALS AND METHODS

In the present study, we have studied the immunogenic potential of prepared rLoa22 protein of local pathogenic *Leptospira* species in a murine model and its ability to induce humoral and cellular immunity was further evaluated by analyzing the IgG subclasses and cytokines produced through immunization.

### RESULTS AND DISCUSSION

Based on the results, mice immunized with rLoa22/adjuvant and a trivalent vaccine induced high titers of total IgG antibodies. All immunized groups were able to increase IgG1 subclass at almost the same level, but in the case of IgG2a subclass, the antibody levels was significantly higher in the vaccine and rLoa22/adjuvant groups than rLoa22 alone.

The animals immunized by the vaccine produced more IL-4 compared to the recombinant groups and also the group that received the rLoa22/adjuvant was higher than rLoa22 alone. The level of IFN- $\gamma$  showed a slight but significant increase in the rLoa22 with and without adjuvant groups. Our results also demonstrated the rLoa22 protein in indirect ELISA, was able to detect the presence of anti-*Leptospira* antibodies in mice serum.

### CONCLUSION

Therefore, the protein can be used either alone or in combination with other leptospiral antigens as a marker in assessing the seroprevalence of leptospirosis and also in development of an effective vaccine against leptospirosis.

**Keywords:** Pathogenic Leptospirosis, Recombinant Loa22 Protein, Immune Responses.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation of phthalates degrading bacteria from activated sludge in petrochemical plant

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Phthalates are synthetic compounds widely used as plasticizers in various industries. Microorganisms, particularly bacteria, have demonstrated the ability to metabolize the hazardous phthalates. Activated sludge; a mixture of microorganisms commonly used in wastewater treatment plants, has been found to harbor diverse microbial communities with the capability to degrade a wide range of pollutants. The aims of this study were isolation and characterization of phthalate degrading bacteria from activated sludge in petrochemical plant.

### MATERIALS AND METHODS

Activated sludge samples were collected from a wastewater treatment plant. About 7 mL of the collect samples was inoculated into 100 mL enrichment medium contains mineral salt medium (MSM) supplemented with 0.5 g L<sup>-1</sup> carbon of each dimethyl phthalate (DMP) or dibutyl phthalate (DBP) as sole carbon and energy source. The flasks were incubated on an orbital shaker at 30 °C, 120 rpm, pH 7±0.2 during 2-7 days. The bacterial degradation activity was determined using a spectrophotometer at 600 nm. The enriched cultures were plated out on selective agar to isolate individual phthalate degrading bacteria. The isolates were further characterized based on morphological, biochemical, and molecular techniques.

### RESULTS AND DISCUSSION

Among 11 isolates, two Gram negative bacteria named MAF1 and MAF11 were degraded both phthalates as a sole carbon and energy source. The results showed that degradation of DMP and DBP by MAF1 and MAF11 were initiated after 12 hours then reached to a maximum after 48 and 120 hours incubation, respectively. In addition, the optical density assay in growth cultures contains DMP and DBP for both isolates were increased from 0.026 to 0.276 and 0.27 to 0.473, respectively.

### CONCLUSION

The results demonstrated two bacteria isolated from activated sludge were able to degrade phthalates. These results revealed that both isolates are suitable for degradation of phthalates in industrial wastewater and contaminated sites.

**Keywords:** Phthalates, degrading bacteria, activated sludge

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# The role of the microbiota in the process of cancer and the future of treatment

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

In spite of decades of clinical research, Cancer has become one of the most important health problems for humans.

Here we review the status of effects of microbiota on the healing process and cancer treatment. The purpose of this review is cancer treatment and reduction of side effects.

### MATERIALS AND METHODS

An organized review was completed on original research extracted from PubMed and Google scholar. We searched with the keywords which is mentioned below.

### RESULTS AND DISCUSSION

By reviewing the data, we came to the conclusion that microbiota metabolites can have an effect on cancer progression and modulate it, and have fewer side effects than radiotherapy. Also, using microbial metabolites in chemotherapy will reduce the severe side effects caused by the treatment and will lead the treatment in a better direction.

### CONCLUSION

The results of present study showed there is possibility that Microbiota has a bright future in cancer treatment, and maybe in the future it can be used as an alternative to radiotherapy and chemotherapy.

**Keywords:** Cancer Therapy, Gut Microbiota, Chemotherapy, Immune System

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# Investigating the antibiotic resistance pattern of *Staphylococcus aureus* isolated from clinical specimens

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Staphylococcus aureus* is the cause of many local and systemic infections. *S. aureus* is known as the main nosocomial pathogen due to its ability to generate a wide range of virulence factors. Examining the pattern of bacterial antibiotic resistance is important to prevent the spread of resistant strains in society. Investigating the antibiotic resistance pattern of *S. aureus* isolated from clinical samples was the aim of the current study.

### MATERIALS AND METHODS

Clinical specimens were collected from local medical labs, directly inoculated on mannitol salt agar, and incubated at 37 °C for 24-48 hours. Isolates were identified as *S. aureus* using colonial morphology, Gram staining, catalase, coagulase and DNase activity tests, and mannitol fermentation test. After identifying the isolated bacteria, phenotypic resistance detection method was used to determine the antibiotic resistance pattern. In addition, oxacillin screening plate method was used for the determination of methicillin resistant isolates.

### RESULTS AND DISCUSSION

The antibiotic susceptibility assay was performed using disc diffusion method. The results revealed that the isolates were resistant to most regular antibiotics, including penicillin (66.6%) and tetracycline (49.9%). In addition, all isolated *S. aureus* were sensitive to gentamicin (100%). Furthermore, the most intermediate resistance was also observed regarding the vancomycin antibiotic (41.6%).

### CONCLUSION

This study indicated that a high percentage of *S. aureus* isolates are resistant to common antibiotics such as penicillin, and their intermediate degree of resistance to glycopeptide antibiotics such as vancomycin is increasing. Therefore, it is important to prevent the unrestrained spread of resistant isolates in the community.

**Keywords:** *Staphylococcus aureus*, antibiotic resistance, methicillin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Assessment of Antibacterial effects of *Silybum marianum* extracts on Extensively drug-resistant *Acinetobacter baumannii*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Acinetobacter baumannii* is one of the most common and important causes of hospital-acquired infections. Due to its intrinsic resistance to antibiotics, *A. baumannii* can survive in the hospital environment for a long time and target hospitalized patients. Therefore, treatment and prevention of patients infected with these bacteria require identification of new antibacterial agents with no or fewer side effects and toxicity. The aim of this study was to investigate and compare the antibacterial effect of aqueous and ethanolic extracts of *Silybum marianum* on extensively drug-resistant *A. baumannii* clinical isolates.

### MATERIALS AND METHODS

Antimicrobial susceptibility of the isolates was determined using the Kirby-Bauer method according to the Clinical and Laboratory Standards Institute document M100 (2020). Antimicrobial activity of the ethanolic and aqueous extracts of *S. marianum* against extensively drug-resistant *A. baumannii* isolates was determined by agar well diffusion method. In addition, broth microdilution susceptibility testing was carried out to determine minimal inhibitory concentrations (MICs) of the extracts. Finally, active compounds with antimicrobial activity were identified by gas chromatography–mass spectrometry.

### RESULTS AND DISCUSSION

Frequency of the extensively drug-resistant, *A. baumannii* isolates was 36.3%. The MIC<sub>90</sub> of the aqueous extract of *S. marianum* was 4096 µg/mL, which was two times less than that of the ethanolic extract (8192 µg/mL). Similarly, the MIC<sub>50</sub> of the aqueous extract of *S. marianum* was significantly smaller than that of the ethanolic extract ( $P < 0.05$ ). According to the results, silybin and silychristin were the most abundant (42.04%) bioactive compounds in the aqueous extract of *S. marianum*.

### CONCLUSION

Given the excellent antibacterial effects of the aqueous extract of *S. marianum*, it is recommended to investigate the potential application of these extracts and their bioactive constituents for production of pharmaceutical compounds and disinfectants.

**Keywords:** *Acinetobacter baumannii*, drug resistance, *Silybum marianum*, Nosocomial infection

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Family detection and dry-tolerance determination of lytic bacteriophage against *Extensively-Drug Resistant (XDR) Escherichia coli*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

phage therapy is one of the effective methods against bacteria that works specific and doesn't have any effect on other hosts. According to this unique feature, using phage as an alternative method instead of antibiotic therapy, would help to solve bacterial problems. The aims of this study were the morphological characterization and family detection of specific bacteriophage against Extensively-Drug Resistant (XDR) *Escherichia coli* and determination of dry-tolerance of it.

#### MATERIAL AND METHODS

A lytic phage against *E. coli* was isolated by standard method and its host range were determined. Phage suspension was condensed and a photo by Transmission Electron Microscopy (TEM) was taken and the viruse family as well as dry resistance for a long time period (10 month) were determined.

#### RESULTS AND DISCUSSION

*XDR E. coli* was lysed by bacteriophage suspension. The bacteriophage was completely specific against *E. coli* and the other strains were no susceptible to it. The bacteriophage had hexagon shape without tail and it was shown that this phage belong to *Podoviridae* family. After one-hour to eight month of incubation of bacteriophage suspension at 45°C in oven, the titers of phage were completely preserved. But at ninth and tenth month the titer was reduced from 10<sup>12</sup> to 10<sup>9</sup>.

#### CONCLUSION

dryness tolerance of this phage was appropriate and it also specifically lysed *E. coli*. It also had great potential for lysing the XDR *E. coli*. According to the results, this phage has a good potential for food preservation, food industry and biocontrol of the dry environment for long period of time, instead of antibiotics or as a companion of them.

**Keywords:** Bacteriophage, Phage Therapy, XDR *E. Coli*, *Podoviridae*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Urinary tract co-infections in patients with papillomavirus

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### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Human papillomavirus (HPV) has been identified the etiologic agent of warts, cervical intraepithelial neoplasia (CIN), and cervical cancer. Among sexually transmitted infections (STIs), only HPV infection is known to be a major cause of cervical cancer.

This virus is a type of DNA virus that mainly affects epithelial cells of the skin and mucous membranes.

HPV virus is divided into high-risk and low-risk types. Most HPV infections, especially the low-risk types, are transient and are eliminated by the host and immune system after a while, but some of these infections can persist and become malignant, which indicates that there are other factors that can have synergistic effect with this virus and cause the transformation of normal cervical epithelial cells into cancer cells.

Aside from HPV, other bacterial infections in the genital tract are associated with cervical neoplasia Such as *Ureaplasma* spp and *Neisseria gonorrhoeae*.

The purpose of this study is to investigate bacterial co-infections with HPV virus and their role in causing cervical cancer in order to reduce Getting this disease.

#### MATERIALS AND METHODS

The studies were conducted as a review of related articles.

#### RESULTS AND DISCUSSION

Researchers believe that the simultaneous infection of different types of HPV virus with some factors such as bacteria can increase the risk of malignancy.

They also found that the presence of some bacteria (*Ureaplasma*, *Chlamydia*, and *Gardnerella*) in the lower part of the female genital system has a positive correlation with the frequency of HPV infection and, consequently, a possible influence on faster progression to cervical dysplasia caused by HPV.

#### CONCLUSION

Researchers Found that the presence of some bacteria (*Ureaplasma*, *Chlamydia*, and *Gardnerella*) in the lower part of the female genital system affect the frequency of HPV infection and, consequently can affect the faster progression to cervical dysplasia.

**Keywords:** Human papilloma virus, Bacterial Coinfection, Cervical cancer.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibacterial Activity of Compounds Derived from Endophytic Bacteria of *Juniperus sabina*: *Enterobacter hormaechei* subsp. *Xiangfangensis* and *Bacillus tequilensis*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Endophytes are microorganisms that live inside plants and have been found to produce biologically active compounds. The study aimed to explore the antimicrobial properties of compounds synthesized by two endophytic bacteria, *B. tequilensis* and *E. hormaechei* subsp. *xiangfangensis*, isolated from the *Juniperus sabina* plant.

### MATERIALS AND METHODS

The isolation of endophytes involved a process of surface sterilization (2.5% sodium hypochlorite and 70% ethanol) followed by cultivation and purification on NA medium. Identification was performed via DNA extraction, amplification of the 16S rRNA region via PCR and sequencing by the Sanger method. To investigate the antimicrobial activity of the isolated bacteria, the supernatant of 7 days culture in minimum media was clarified by centrifugation at 12000 rpm, 4 °C for 20 minutes and dried using a rotary evaporator at 40 °C. Desalting and extraction was performed by methanol (1:10, W/V) and extracted materials were dried and kept at 4 °C. Thereafter, well diffusion method was followed by the determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) according to CLSI protocol with some modifications, against four bacterial standard strains.

### RESULTS AND DISCUSSION

Chromaspro software was utilized to edit the sequenced genes. Sequences were blasted on the NCBI and EzBioCloud sites to identify the bacteria. Finally, genes of the two identified bacteria were submitted to the NCBI site. According to the results of well diffusion method supernatant of *B. tequilensis* and *E. hormaechei* subsp. *xiangfangensis*, cultures could inhibit *Bacillus subtilis* ATCC 6051 and *Escherichia coli* ATCC 11775 (20 and 15 mm for 10 mm diameter wells, respectively). While the methanolic extract of *B. tequilensis* showed MIC values of 12.5 and 25 mg/ml against *S. aureus* and *B. subtilis*, sample of *E. hormaechei* subsp. *xiangfangensis* demonstrated a MIC value of 25 mg/ml against *S. aureus*. *Salmonella typhi* PTCC 1609 could not be inhibited by evaluated samples.

### CONCLUSION

The study showed a mild antibacterial activity of two endophytic bacteria isolated from the *J. sabina* plant, and determination of effective antibacterial compound (s) will be performed in ongoing analysis with RP-HPLC in parallel with antibacterial assessment of purified compounds.

**Keywords:** *Juniperus sabina*, Endophyte, 16S rRNA, PCR, MIC, MBC

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of diazinon pesticide biodegradation efficiency by native bacterial strains isolated from contaminated soils in agricultural areas

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Diazinon is one of the most widely used organophosphorus pesticides in agriculture. The accumulation of this pesticide in agricultural soils and its intrusion into water sources pose a risk to public health and the environment. The aim of this study was to investigate the efficiency of bioremediation of diazinon pesticides by indigenous bacteria isolated from contaminated soils.

### METHODS AND MATERIALS

Soil samples were collected from a tomato greenhouse in Meybod city. The soil samples were cultured in a mineral nutrient medium containing 10% (v/v) diazinon as the sole carbon source. In order to adapt the isolates to higher concentrations, culture media containing 20-70% diazinon were used in the next experiments. The growth rate of the isolates was determined by measuring the optical density of the samples with a spectrophotometer at a wavelength of 620 nm. The synergistic effect of the isolates in the biodegradation of diazinon was also investigated. Superior isolates were identified by biochemical and polymerase chain reaction (PCR) assays. The amount of the pesticide diazinon remaining in the culture medium was measured by gas chromatography (GC) with flame ionization detector (FID).

### RESULTS AND DISCUSSION

Culture of the isolates showed that there were two superior isolates in the soil samples that could grow in a saline medium containing 60% (v/v) diazinon. The results of biochemical tests and partial 16SrRNA gene sequence analysis and blast showed that the two best strains belonged to *Pseudomonas aeruginosa* and *Entrobacter huaxiensis* with 94.5% and 94.7% similarity, respectively. The results of GC analysis showed that *Ps. aeruginosa* and *E. huaxiensis* were able to degrade 87.45% and 75.04% of diazinon, respectively. Simultaneous cultivation of these two species in the culture medium containing diazinon showed no synergistic effect and resulted in the degradation of only 44.34% of diazinon.

### CONCLUSION

The species of *Ps. aeruginosa* and *E. huaxiensis* are suitable candidates for the biodegradation of diazinon in contaminated soils. Biodegradation efficiency decreases in the presence of both bacteria, probably due to the release of secondary metabolites. In order to increase the biodegradation efficiency of diazinon, physical and photocatalytic solutions can be considered along with the use of bacteria.

**Keywords:** Biodegradation, Diazinon, Pesticide, *Pseudomonas aeruginosa*, *Entrobacter huaxiensis*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of antibiotic resistance of uropathogenic *Escherichia coli* isolates obtained from Afzalipour hospital in Kerman, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Uropathogenic *Escherichia coli* (UPEC) is the primary cause of urinary tract infection (UTI) in developed countries such as Iran. Important way to control acute urinary tract infections is the accurate antibiotic therapy. An increasing resistance rate relative to the antibiotics recommended by current guidelines for the treatment of UTIs and an increasing number of multidrug resistant UPEC isolates were observed in recent years. Accordingly, the aim of this study was to evaluate the antibiotic resistance of uropathogenic *Escherichia coli* (*E. coli*) isolates.

### MATERIALS AND METHODS

Uropathogenic *Escherichia coli* were isolated from clinical samples obtained from Afzalipour hospital of Kerman city during spring of 2023. Standard biochemical tests were used for identification of UPEC. Twelve common antibiotics from different groups were also selected and used. The antibiotic resistance was determined by Kirby-Bauer disk diffusion susceptibility test. The size of the clear zone around the antibiotic disc is used to classify the antibiotic test results as sensitive, intermediate or resistant. Lack of susceptibility to at least each pathogenic agent in three or more chemical classes of antibiotics was recognized as multidrug-resistant (MDR) *E. coli*.

### RESULTS AND DISCUSSION

A total of 30 UPEC strains were isolated from all clinical samples. All identified isolates were fermentative gram-negative motile rods with metallic sheen on EMB, positive for catalase, indole and nitrate reduction but negative for oxidase, citrate, urease and gelatin. resistance of isolates to the common antibiotics was observed as Ampicillin (76%), Ciprofloxacin (70%), Cefazolin (73%), Trimethoprim/sulfamethoxazol (70%), Imipenem (43%), Cefotaxime (70%), Ceftazidime (53%), Cefepim (46%), Gentamicin (13%), Meropenem (13%), Nitrofurantion (10%), Amikacin (0%). Multidrug resistance (MDR) was also observed as 56.7%.

### CONCLUSION

MDR UPEC has become a complex problem in clinical treatment, and it is essential to monitor *E. coli* resistance from different sites of infections such as UTI. Updated antibiotic resistance data may enable clinicians to have a better recommendations about antibiotic treatments for UTIs.

**Keywords:** *Escherichia coli*, urinary tract infection, antibiotic resistance, Multidrug resistant.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The association between the virulence genotypes of *Helicobacter pylori* strains and the degree of their adhesion to the human gastric epithelium, MKN45 cells

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Helicobacter pylori* (*H. pylori*) is a bacterial class-I carcinogen that specifically colonizes the gastric epithelium of humans as a unique niche. The adhesion of *H. pylori* to the gastric epithelium is not only crucial for successful colonization and pathogenesis but also essential for invasion into host cells. The study aimed to analyze whether *H. pylori* isolates carrying diverse virulence genotypes present an association in the rate of adherence to gastric epithelial cells (MKN45).

#### MATERIALS AND METHODS

Nineteen *H. pylori* strains with defined allelic variants of virulence factors, *cagA*, *vacA*, *iceA*, *babA2* and *sabA*, were selected from the microbial collection of Foodborne and Waterborne Disease Research Center, Shahid Beheshti University of Medical Sciences. The response of *H. pylori* adherence to the MKN45 cell line was analyzed after incubation for 3 h. The host MKN-45 cells were then lysed (brain heart infusion (BHI) broth, 37 °C, 15 min) and adherent bacteria were counted on Brucella agar plates supplemented with 10% horse serum and 7% horse blood (microaerobic atmosphere, 37 °C, 7 days), and then CFUs were enumerated. Finally, the correlation between the adhesion of studied strains to the MKN45 cell line and their virulence genotypes was analyzed by SPSS statistical version 17.

#### RESULTS AND DISCUSSION

A wide range of adhesion potential was found among the studied *H. pylori* strains. The comparison of adhesion indices revealed that the greatest adherent strain to MKN-45 cells was OC291 (41250 CFU/well), followed by HC136 (27 CFU/well), and OC309 (27 CFU/well) as the lowest ones. There was no significant association between the adhesion index of studied strains and their virulence factors such as *cagA*, *vacA*, *iceA*, and adhesins (*babA2* and *sabA*) (*CagA*+ 79 % , *CagA*- 21% , *vacA s1m2* 68% , *vacA s2m2* 26% , *vacA s1m1* 5.2 / *ice A1+A2* 47% , *ice A1* 36.8% , *ice A2* 5.2% , *ice A1-* 10.52% / *sabA*+ 94.7% , *sabA-* 5.26% / *babA*+ 100%.

#### CONCLUSION

Considering, the results indicated that there was no significant association between the adhesion index of studied strains and their genotype of *cagA*, *vacA*, *iceA* and adhesins (*babA*, *sabA*). So, other virulence factors may be more important in the adhesion of *H. pylori* to the human gastric carcinoma cell line (MKN45).

**Keywords:** *Helicobacter pylori*, adherence assay, Virulence factor genotyping

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation of antibacterial activity of copper nanoparticles on *Staphylococcus aureus* isolated from clinical samples in Kerman hospitals

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## ABSTRACT

## BACKGROUND AND ABJECTIVE

*Staphylococcus aureus* is one of the most important bacteria causing hospital infections, which has become resistant to various antibiotics over time. *Staphylococcus aureus* is a gram-positive bacterial that can cause disease in humans. Nanoparticles can penetrate into the cracks of small molecules and cause disintegration in them. Therefore, nanoparticles can be considered a new generation of antibiotic. The aim of this study was to investigate the therapeutic effect of copper nanoparticles on *Staphylococcus aureus* isolated from clinical samples, considering the growth of antibiotic resistance and exploring new approaches in Nano biotechnology.

## MATERIALS AND METHODS

In this study, 30 strains of *Staphylococcus aureus* isolated from clinical samples were examined based on catalase, coagulase, and mannitol fermentation tests for identification and diagnostic purposes. The antibacterial effect of copper nanoparticles synthesized by chemical method was investigated in different concentrations on *staphylococcus aureus* isolates was investigated by disk method. After incubation at 37 °C for 24 hours, the sensitivity of bacteria was determined by measuring the diameter of the growth inhibition zone, and the minimum inhibitory concentration by micro titer plate method was determined.

## RESULTS AND DISCUSSION

66% of *Staphylococcus aureus* isolates were sensitive to the antibiotic gentamicin, and the average minimum inhibitory concentration was found to be 128 µg/ml.

All isolates of *Staphylococcus aureus* showed sensitivity to copper nanoparticles, and the average minimum inhibitory concentration was obtained 64 µg/ml.

## CONCLUSION

The sensitivity of *Staphylococcus aureus* to copper nanoparticles is higher than to antibiotics. The effectiveness of copper nanoparticles against clinical strains of *Staphylococcus aureus* indicates that this substance can be considered as a suitable antimicrobial agent. it is suggested More research should be done.

**Keywords:** *Staphylococcus aureus*, copper nanoparticles, antibacterial

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The Impact of COVID-19 on Medical Microbiology

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The outbreak of COVID-19, caused by the novel coronavirus SARS-CoV-2, has had a significant impact on various aspects of our lives. One area that has experienced a paradigm shift in research and diagnostics is medical microbiology. The COVID-19 pandemic has prompted a shift in research priorities within medical microbiology. Many researchers and microbiologists have directed their efforts towards studying COVID-19, including understanding the virus, its transmission dynamics, and the development of diagnostics, treatments, and vaccines.

The COVID-19 outbreak has shown us new ways to enhance our understanding of how to handle a new species of virus. We want to study the impact of COVID-19 on medical microbiology and explore new methods of organizing information.

### MATERIALS AND METHODS

We utilize a compilation of information that researchers have obtained using diverse materials and methods such as patient samples, Cell Culture, Genomic Sequencing, Bioinformatics, Data Analysis and others. The combination of these materials and methods has facilitated a comprehensive understanding of the impact of COVID-19 on microbiology.

### RESULTS AND DISCUSSION

The COVID-19 pandemic has had a profound impact on medical microbiology. It has led to increased demand for COVID-19 testing, disruptions in routine microbiology testing and temporary shift in research priorities. Understanding these impacts will help in better managing the challenges faced by medical microbiology during and beyond the pandemic.

### CONCLUSION

The developments spurred by the pandemic will not only improve our ability to tackle COVID-19 but will also have a lasting impact on our understanding and management of other infectious diseases. Moving forward, it is essential to sustain the momentum of research and investment in medical microbiology to be better prepared for future public health crises.

**Keywords:** COVID-19, Impact, Medical microbiology

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigation of the most common bacterial infection in hospital wounds

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

Usually, infections that appear in the patient's body 48 to 72 hours after the patient's admission to the hospital are considered hospital infections. *Pseudomonas aeruginosa* bacteria have a high prevalence rate in various types of wounds, such as burns, bedsores, diabetic foot ulcers, etc. Hospital-acquired infections resulting from these wounds are a major cause of morbidity and mortality in patients. The aim of this study is to investigate the, during the years 1401 and 1402 in Motahhari burn hospital and Firuzabadi Hospital in Rey city.

##### MATERIALS AND METHODS

In the conducted study, all 200 patients of Motahhari Burn Hospital and Firuzabadi Hospital in Rey, between the years 1401 and 1402, were examined and studied. They were evaluated based on the type of wound (burn, bed sore, diabetic ulcer), wound grade, wound depth, infection quantity, age, gender, wound percentage and quantity, duration of hospitalization, and the season under investigation.

##### RESULTS AND DISCUSSION

Out of 200 patient samples, 48 patients (24%) were found to have *Pseudomonas aeruginosa* infection. The amount of *Pseudomonas aeruginosa* infection increased during the spring and summer seasons when the weather was warmer. The rate of *Pseudomonas aeruginosa* infection was higher in bedsores (40.8%) compared to burns (30.2%), and higher in burns compared to diabetic ulcers (29%).

##### CONCLUSION

Based on the conducted studies and research, adherence to standard infection control principles is essential in hospitals. Factors such as wound type, season, and the prevalence of *Pseudomonas aeruginosa* should be considered in hospital infection control programs.

**Keywords:** *Pseudomonas aeruginosa*, hospital-acquired infection, burn wound, bedsores, diabetic foot ulcers.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The Influence of Pectin Dietary Fiber on Total Phenolic, Total Flavonoid Content and Antioxidant Capacity of Milk Fermented by *Lactobacillus Paracasei* PTCC 1942

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Today, there is an increasing interest in developing of fiber-enriched functional foods and in the use of natural food as health improving additives. Pectin is a polysaccharide which was added in food as an additive and dietary fiber. Pectin's are composed of significant bioactive compounds. One the other hand, fermentation is a good processing technique due to the enhanced retention of phytochemical contents. Hence, the present study aimed to evaluate the impact of citrus pectin on the total phenolic (TPC), total flavonoid (TFC) content and antioxidant capacity of milk fermented by *L. paracasei* for producing fiber-enriched functional food.

### MATERIALS AND METHODS

For production of fermented milk, *L. paracasei* PTCC 1942 strain was inoculated (1% v/v) into milk. At the end of fermentation, the pectin was added (1% w/v) to the fermented milk. Water soluble extract (WSE) of fermented milk was separated. The TPC and TFC were determined through Folin-Ciocalteu reagent and aluminum chloride methods, respectively. The antioxidant capacity was measured by inhibition of 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays.

### RESULTS AND DISCUSSION

Our findings indicated that the DPPH scavenging rate (from 41.5 to 78.5%), TPC (from 94.45 to 123.33  $\mu\text{gr GAE/mL}$ ), and TFC (from 341 to 359.5  $\text{QE } \mu\text{gr QE/mL}$ ) were increased with the addition of pectin to WSE ( $p < 0.05$ ). In contrast, the addition of pectin to WSE reduced free radical scavenging ABTS (from 85.93% to 74.43%) ( $p < 0.05$ ).

### CONCLUSION

Based on the findings, it can be concluded that the addition of pectin can be helpful to produce fermented milk with enough rich in functional properties.

**Keywords:** Pectin, Fermented Milk, Bioactive compound, Fiber-Enriched Functional Food

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Influence of Fructooligosaccharide on Oxidative Stress, and Inflammation as a Preventive Agent Type 2 Diabetes in-Vitro

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Diabetes mellitus is a metabolic disease and inflammatory condition with hyperglycemia. Hyperglycemia promotes the production of free radicals that leads to inflammation. There is a strong link between hyperglycemia, hyperglycemic-induced oxidative stress, inflammation and the development and progression of type 2 diabetes. Therefore, the roles of anti-inflammatory agents, and antioxidants in treating diabetes should not be under-estimated. Recently, prebiotics are known as new antidiabetic agents. There are few data about hypoglycemic effects of prebiotics especially for fructooligosaccharide (FOS). Therefore, main aim of present study was evaluation of the influence of FOS on oxidative stress, and inflammation in-vitro

### MATERIALS AND METHODS

At first, the effect of FOS on the activities of  $\alpha$ -amylase,  $\alpha$ -glucosidase and glucose transport across yeast cell membrane was evaluated. Then, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azinobis 3 ethylbenzothiazoline-6-sulfonic acid (ABTS) assay were employed to determine its antioxidant effects. The total phenolic (TP) and total flavonoid (TF) content were evaluated by colorimetric assay. The anti-inflammatory effect was determined using egg albumin denaturation.

### RESULTS AND DISCUSSION

Our findings showed that the FOS has the potential to inhibit  $\alpha$ -glucosidase enzyme. In addition, yeast cell glucose uptake study revealed inhibition of glucose absorption 15% with respect to control ( $p < 0.05$ ). FOS indicated the ability to inhibit DPPH free radical with TP and TF content in a concentration-dependent manner. The inhibition rate of protein denaturation was obtained to be 20% at a dose of 10 mg/mL.

### CONCLUSION

According to the findings, it can be concluded that antidiabetic, antioxidant, anti-inflammatory properties of FOS could be exploited as a new tool against diabetes. Thus, further study of FOS for the development of antidiabetic agents is warranted.

**Keywords: Diabetes, Prebiotic, Fructooligosaccharide, Oxidative stress, Inflammation**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### First Report of Outbreak of Nosocomial Sepsis caused by *Pantoea* spp. among NICU patients in Northeast of Iran

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

*Pantoea* is an environmental gram-negative bacillus that rarely causes an opportunistic infection, especially bloodstream infections in NICU. Here we report bloodstream infections caused by *Pantoea* spp. in 5 preterm neonates in the NICU and this is the first documented outbreak from the northeast of Iran.

#### MATERIALS AND METHODS

On July 2023-26-6, an outbreak was created in the NICU of Ghaem Mashhad Hospital. After identifying the outbreak, sampling was done from all parts of the NICU to identify the source of infection. after the investigation, the source of infection was introduced as the index case was born from an addicted mother at 26 weeks. There was fecal contamination during delivery, which was not washed for several hours because the baby was premature. The baby was transferred to NICU from birth and expired after 48 hours. The blood culture of these neonate was done in BACTEC. Initial identification was done with routine biochemical tests, and for more accurate identification, we used the VITEC2 device. The second baby was born at the same time as the first baby, and the next three babies were present when the first baby arrived in the NICU, and the infection was transmitted through the hands of nurses and contaminated equipment. Supportive measures led to quick and timely control of the outbreak.

#### RESULTS AND DISCUSSION

Three of the five neonate expired. Identification was done with routine biochemical methods and Vitek2 device, and finally, the genus *Pantoea* spp. was reported. The blood cultures of all five babies were reported to be positive for *Pantoea*. The disease progressed quickly in baby. Therefore, quickly actions to control this outbreak were important. Infection control was done in a short period of time with quick diagnosis and effective measures by hospital staff.

#### CONCLUSION

Based on the evidence of the microbiological investigation, the source of infection was probably the index case. Finally, the infection was controlled in a short time with the quickly actions of the hospital staff, including the isolation of infected infants, the quick washing of the NICU along with all the tools and equipment with very strong disinfectant solutions.

**Keywords:** First report, NICU, Outbreak, Neonate

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Genotypic and Phenotypic Investigation of Antibiotic Resistance of *Salmonella* Strains Isolated from Clinical Specimens in Mashhad Teaching Hospitals

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

Salmonellosis is a major water and food-borne infection worldwide. With high mortality rates particularly among young children, antimicrobial resistant salmonellosis is considered a public health issue in low-income countries. Therefore, the aim of this study was to evaluate the genotypic and phenotypic resistance of third-generation cephalosporins and carbapenems in *Salmonella* strains isolated from clinical samples in Mashhad teaching hospitals.

### MATERIALS AND METHODS

In this cross-sectional study, 64 isolates of *Salmonella* were obtained from stool, blood, and urine samples of patients in teaching hospitals in Mashhad. Biochemical tests were used to identify and confirm the genus. In addition, *Salmonella* antiserum kits were used to diagnose different serotypes and serogroups of pathogenic *Salmonella*. The antimicrobial resistance pattern for different antibiotics, including third-generation cephalosporins and meropenem, was determined by the disk diffusion method according to CLSI guidelines. The presence of ESBL, AmpC, and carbapenems encoding genes was evaluated using PCR and sequencing.

### RESULTS AND DISCUSSION

*Salmonella* isolates were serotypes A (n=5, 7%), B (n=6, 10%), C (n= 13, 21%), and D (n= 40, 62%). Antibiogram results showed that the most active antibiotics against these bacteria were meropenem (100%), gentamicin (97%), and chloramphenicol (94%). Also, the highest antibiotic resistance rates were found for ampicillin (20%) and trimethoprim-sulfamethoxazole (21%).

The PCR results showed that 6 isolates were positive for ESBLs-producing genes, that *bla*<sub>CTX-M-15</sub> and *bla*<sub>TEM</sub> were identified in all 6 samples and *bla*<sub>SHV</sub> was identified in one of them. In addition, multiplex-PCR results demonstrated that 4 isolates (3 of which were ESBL positive) produced AmpC  $\beta$  lactamases, that *bla*<sub>CMY</sub> and *bla*<sub>LAT</sub> genes were identified in all 4 samples and *bla*<sub>FOX</sub> was in 3 of them. Due to the lack of identification of meropenem-resistant isolates, PCR was not performed to identify the genes responsible for carbapenem resistance.

### CONCLUSION

The phenotypic and genotypic results found in this study highlight the need for surveillance of antibiotic resistance and screening for ESBL production to support appropriate action.

**Keywords:** *Salmonella*, ESBL, AmpC, Antibiotic susceptibility

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Frequency of the *Bacteroides fragilis* Toxin Gene Subtypes in colorectal cancer (CRC) patients and outpatient colonoscopy for CRC screening

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The enterotoxigenic *Bacteroides fragilis* (ETBF) is identified with acute infections, inflammatory bowel disease, and especially colorectal cancer (CRC). This study aims to understand the prevalence and variations of enterotoxin types in *Bacteroides fragilis* strains isolated from CRC patients, as these strains are known to promote colon carcinogenesis in experimental setups.

#### MATERIALS AND METHODS

This study compares the presence of the *bft* gene in fecal samples collected from 21 colorectal cancer patients (cases) and 43 individuals undergoing outpatient colonoscopy for CRC screening or diagnostic purposes (controls). The presence of the *bft* gene in individual bacterial colonies, isolated from stool samples under anaerobic conditions, was tested using the 16S rRNA polymerase chain reaction.

#### RESULTS AND DISCUSSION

In both tumor and/or normal tissues, a high frequency of *bft*-positivity (85.7%) was observed in the stool of the cases. Concordance in detection of the *bft* gene was noted in most paired stool samples from individual cases (76%) or controls (68%). Additionally, there was a trend towards higher *bft* positivity in mucosa from late-stage as compared to early-stage CRC patients. Unlike ETBF diarrheal disease, where *bft*-1 detection is dominant, *bft*-2 was the most commonly identified toxin isotype in both cases and controls.

#### CONCLUSION

This study suggests that the *bft* gene is associated with colorectal neoplasia, particularly in advanced stages of CRC. This study provides greater insight into the potential role of *Bacteroides fragilis* in colorectal cancer development and may pave the way for focused treatment methods in the future.

**Keywords:** enterotoxigenic *Bacteroides fragilis*, colorectal cancer, enterotoxin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Biofuel Production by Cyanobacteria Utilizing Industrial CO<sub>2</sub> Emissions

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The substantial combustion of fossil fuels for energy generation leads to the release of excessive air pollutants, particularly carbon dioxide. In light of this, exploring alternative approaches involving solar energy utilization and harnessing CO<sub>2</sub> emissions from industries through photosynthesis holds promise for reducing air pollution. Cyanobacteria, as photosynthetic microorganisms, exhibit remarkable potential across various applications, with a particular emphasis on biofuel production.

### MATERIALS AND METHODS

This research was conducted in a systematic manner.

### RESULTS AND DISCUSSION

This study investigates the advantages of utilizing cyanobacteria, photosynthetic bacteria that use water as an electron donor and carbon dioxide as a carbon source during photosynthesis, for biofuel production. The research focuses on the effective factors influencing cyanobacteria cultivation, the optimization of photobioreactors to create a suitable biochemical environment, the biofuel production process, and the valuable by-products generated as a result.

### CONCLUSION

Cyanobacteria, being photosynthetic microorganisms abundant in nature, have garnered significant attention for biofuel production due to their simple nutrient requirements and shorter generation time. In the cultivation of cyanobacteria within photobioreactors, it is crucial to optimize carbon dioxide gas delivery, light distribution, and mass transfer systems to achieve higher biomass yields. Subsequent to biomass cultivation, the biofuel extraction process follows. Additionally, valuable co-products are generated during this process, further enhancing the economic viability. Furthermore, the potential use of sewage and sea water for cyanobacteria cultivation adds to the economic appeal of this approach.

**Keywords:** Cyanobacteria, biofuels, carbon dioxide, photobioreactors.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The effect of different cooking methods on the microbial properties of rainbow trout

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## ABSTRACT

## BACKGROUND AND OBJECTIVES

Thermal processing techniques are widely used to improve eating quality and safety of food products and to extend the shelf life of the products. Fish products are cooked in different ways to improve its hygienic quality by inactivation of pathogenic microorganisms and to enhance its flavor and taste. The rainbow trout is probably the most widely introduced fish species in the world. The purpose of this study is to determine the effect of different cooking methods on the microbial characteristics of rainbow trout.

## MATERIALS AND METHODS

For this purpose, ten rainbow trout fish with a mean weight  $700 \pm 100$  g were purchased from the fish market in Rasht. The studied groups were included, group(control): raw fish, group 2: grilled fish, group 3: boiled fish and group 4: fried fish. All the samples were manually filleted and then washed and grilled, boiled and fried using frying oil. Microbial characteristics including total microbial count, total coliform count, detection of *Escherichia coli* and coagulase positive *Staphylococcus*, mold and yeast count were determined according to Iranian national standards method.

## RESULTS AND DISCUSSION

The results showed that cooking methods lead to significant changes in the amounts of microbial load, but no difference was seen in the germicidal ability of different cooking methods in this study. Among the tested methods, the boiling method has shown the lowest microbial load.

## CONCLUSION

Different cooking methods can increase the safety of food from a microbial point of view, but they can differ in terms of nutritional properties.

**Keywords:** Cooking methods, Microbial characteristics, rainbow

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The Interplay between Oral Microbiota and Lung Cancer: A Review

Yasaman Alirezaei

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Lung cancer is a leading cause of cancer-related deaths worldwide. The role of the oral microbiota has recently been highlighted as a significant factor implicated in lung cancer pathogenesis. Lung cancer is a major global health burden. Luckily, oral microbiota has begun to emerge as a potential contributor to the etiology of lung cancer.

### MATERIALS AND METHODS

Studies were identified by searching PubMed, Scopus, and Web of Science databases up until June 2023, using the keywords "oral microbiota", "lung cancer", "microbiome", and their variants.

### RESULTS AND DISCUSSION

Out of 382 identified articles, 25 met the inclusion criteria and were included in this review. Several studies reported Lung cancer patients showed increased diversity and richness in their oral microbiota. Notably, the abundance of certain bacterial taxa, including *Porphyromonas gingivalis*, *Prevotella* and *Veillonella*, was significantly higher in lung cancer patients. *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, common oral pathobionts, have been linked with lung cancer. They can translocate to the lungs, cause chronic inflammation, and disrupt the immune response. *Porphyromonas gingivalis* has been found to induce cellular proliferation and inhibit apoptosis, thereby promoting tumorigenesis. Periodontal disease, an indicator of altered oral microbiota, has been associated with increased lung cancer risk. Chronic inflammation, systemic dissemination of oral pathogens, and immune dysregulation are potential mechanisms through which poor oral health might influence lung carcinogenesis. Altered oral microbiota has been consistently reported in lung cancer patients. It suggests that oral microbiota might play a crucial role in lung carcinogenesis through chronic inflammation and immune modulation. Thus, novel therapeutic strategies could be developed based on these findings. Modulating oral microbiota via probiotics, prebiotics, or antimicrobial therapy might contribute to lung cancer prevention and treatment.

### CONCLUSION

This review underscores the potential role of oral microbiota in lung cancer. Future studies should focus on unraveling the mechanisms involved in the interaction between oral microbes and lung cancer cells.

**Keywords:** Oral\_Microbiota, Lung\_Cancer, *Porphyromonas\_Gingivalis*, *Fusobacterium\_Nucleatum*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of the effect of *Lactobacillus planetarium* probiotics produced from broad bean seed in prevention of *Helicobacter pylori* in stomach tissue of C57BL/6 mice

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Helicobacter pylori* is one of the most common human infections, which colonizes more than half of the world's population. This causes chronic stomach inflammation diseases without clinical syndromes, gastric and duodenal ulcer, and stomach cancer. Nowadays, the use of probiotics has received much consideration as one of the common therapeutic methods, which prevents bacterial colonization by creating a balance in the microbial gastrointestinal tract.

### MATERIALS AND METHODS

This experimental study was conducted on 30 rats in five groups from August 2016 to June 2017 in the Microbiology and Animal Laboratory of Shahrekord University. First, the rats were infected with *H. pylori* bacteria. Polymerase chain reaction (PCR) method was used to confirm the presence of bacteria in the stomach to ensure that the rats were inoculated with *H. pylori*. After inoculation, the infected rats were treated with the probiotic product, and then gastric tissue of the infected group was evaluated by the hematoxylin and eosin stain.

### RESULTS AND DISCUSSION

The absence of *Cag A* and *Ure C* genes in fecal specimens of the group receiving probiotic products before and after *H. pylori* incubation showed a positive effect for this product on the prevention and treatment of *H. pylori* infection. Also, in stomach histology specimens, the effects of mild inflammation were observed in the treated group with the probiotic product before and after *H. pylori* inoculation compared to the control group.

### CONCLUSION

The results of this study showed that the addition of probiotic to a non-dairy product (broad bean extract) can be effective in preventing and treating *H. pylori* infection in the animal model.

**Keyword:** *Helicobacter pylori*, Probiotic, Broad bean, Haematoxylin, Gastrointestinal tract

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Probiotics role in the detoxification of heavy metals in food

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Heavy metals such as Pb, Cd, As, and Cr in the Earth's crust are toxic at low concentrations. They enter our body and Food via water, air, and soil. Heavy metal toxicity can damage the function of important organs and physical, muscular, and neurological degenerative processes. Repeated long-term exposure of some metals and their compounds may even cause cancer. Probiotics are viable microorganisms that show health benefits if presented in sufficient amounts. This article reviews the role of probiotics in the reduction and absorption of heavy metals in food.

#### MATERIALS AND METHODS

A literature survey with the keywords such as probiotics, detoxification, heavy metals, and food was conducted in the following databases: Google Scholar, Pub Med, and Elsevier (2018-2022), and 50 articles, FAO and WHO reports were selected. All of them mention the effects of probiotics, especially their role in detoxification of harmful compounds such as heavy metals in food.

#### RESULTS AND DISCUSSION

It was found that Metal toxicity depends on the absorbed dose, the route of exposure and duration of exposure and various public health measures have been undertaken to control, prevent and treat metal toxicity. Probiotics can reduce the toxicity of toxic substances such as mycotoxins, heavy metals, or harmful chemicals in food packaging through the binding and absorbing them. The merits of probiotics or potential probiotics for bioremoval are being: directly applicable in foodstuffs, simple bioactivity, economical, cheap and fast.

#### CONCLUSION

Lactobacillus, Bacillus, Bifidobacterium and Saccharomyces cerevisiae are the most prominent probiotics. Food contaminants bioremoval is a green technology to control many health risks. Some probiotics are proven for being able to remove heavy metals from foods. The most effective probiotics is better to be applied as biosorbant in contaminated food to enhance food safety. More assessments are needed to discover the bioremoval mechanism of probiotics in food.

**Keywords: Probiotics, Heavy metals, Bio removal, Food.**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Rabies and Public Health: The Importance of Rabies and Rabies Control Strategies

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Rabies is a viral zoonotic disease which causes fatal encephalitis. Rabies is one of the most important public health concerns in many endemic countries like Iran which suffer a lot of economic losses, especially in public health system. Rabies cases are caused by dogs (99%); Studies show that it is possible to eliminate or reduce dog rabies. This study reviewed the importance of rabies and rabies control strategies that have been implemented in different countries.

### MATERIALS AND METHODS

A literature survey with the keywords: rabies, control, rabies elimination, and strategies were conducted in databases: Google Scholar, Pub Med, and Elsevier (2018-2022), and 21 articles and WHO reports were selected. All these articles introduce examination methods, pathogenesis, and efficient rabies control strategies.

### RESULTS AND DISCUSSION

Investigation of rabies control methods showed that rabies is endemic in countries with a lack of rabies pre and post-exposure prophylaxis centers and improper rabies control in free-roaming/stray dogs and wildlife, improper dog population management, lack of diagnosis, and labor. The important methods of rabies control, which have led to the elimination of dog-mediated rabies in Western European countries, Canada, USA, and Japan, are continuous coverage of animal vaccination (dog vaccination coverage of 70%) and wildlife, inter-sectoral cooperation, dog population control, establishing rabies diagnosis centers, allocating funds, monitoring the rabies control program, increase public awareness and education, increasing health centers, and rapid reporting.

### CONCLUSION

Considering the increase in animal bite cases in Iran in recent years and the fatality of rabies, studying the rabies and rabies control strategies are very important. We hope to eliminate rabies in the country by designing and developing national plans. Implementation of the global strategic plan to control rabies make it possible to progress in eliminating dog-mediated rabies.

**Keywords:** Animal bite, Rabies elimination, Public Health, Iran.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Prevalence of Methicillin Resistance and Some of Virulence Genes in Clinical Isolates of *Staphylococcus aureus*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Staphylococcus aureus* is one of the most important pathogens that produces a wide range of toxins and plays a role in causing disease in the host. The aim of this study was to identify prevalence of virulence genes and antibiotic resistance patterns among different clinical isolates of *S. aureus*.

#### MATERIALS AND METHODS

In this cross sectional and descriptive study, clinical isolates of *S. aureus* were collected from three referral hospitals in Sari city and identified by using Standard biochemical tests. To determine the antibiotic resistance pattern, Kirby-Bauer disk diffusion method and minimum inhibitory concentration (MIC) were conducted. All isolates were screened for *mecA*, enterotoxin A (*SEA*), enterotoxin B (*SEB*) and Alpha-hemolysin (*HLA*) genes. Statistical correlations were carried out.

#### RESULTS AND DISCUSSION

A total of 112 *S. aureus* isolates were identified, which were subjected to antimicrobial susceptibility testing. A total of 67 (59.8%) strains were found to be MRSA isolates which were cefoxitin MIC of >4 µg/ml and resistant to methicillin. The highest frequency of antibiotic resistance was observed against erythromycin in 61 isolates (91%) and clindamycin in 48 isolates (71.6%) the lowest resistance was against in norfloxacin 9 isolates (13.4%) and amikacin in 14 isolates (20.9%). MRSA isolates showed 100 and 0% resistance to cefoxitin and vancomycin, respectively. *mecA* genes were present in all MRSA isolates. *HLA* was found in 37.3% of MRSA isolates. *SEA* genes were detected in 23.8% MRSA isolates while the *SEB* gene recovered from only 2.9% MRSA isolates.

#### CONCLUSION

The results illustrated the diversity of antibacterial resistance and toxin gene profiles among MRSA isolates. The increased prevalence of methicillin-resistant *S. aureus* isolates containing virulence genes and antibiotic resistance genes is a serious threat for the hospitalized patients. The results showed that the relationship between virulence genes and antibiotic resistance pattern can be considered as an important issue.

**Keywords:** Alpha-hemolysin, enterotoxin A, enterotoxin B, *mecA*, *Staphylococcus aureus*, MRSA.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Common typing methods based on molecular approaches of *Listeria monocytogenes* - MLST, PFGE, WGS

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Listeria monocytogenes* is a gram-positive foodborne pathogen causes listeriosis which appears from self-limiting gastroenteritis to severe invasive infections. This bacterium can survive in a wide range of temperatures and pH, so its identification is important. Considering multiplicity of investigation methods and the importance of identification of this bacterium, this study aims to show the importance of molecular investigation in today's advanced world and compare the three common molecular typing methods, MLST, PFGE and WGS.

#### MATERIALS AND METHODS

From the collection of 114 articles in the field of *Listeria monocytogenes* and its subgrouping method from 2018 to 2023 found through some of the international and internal databases such as PubMed, google scholar, SID and Civilica, 34 of them were used in this article.

#### RESULTS AND DISCUSSION

The results showed the unique characteristics of each method. By examining three widely used methods of molecular typing, including the Pulse Field Gel Electrophoresis method, which was once known as the gold standard for typing bacterial pathogens, as well as the Multi-Locus Sequence Typing method, which is one of the most commonly used methods and can identify large number of *Listeria monocytogenes* sequence types, with the method based on whole genome sequencing, more details of molecular characteristics can be obtained.

#### CONCLUSION

Due to the increasing progress of laboratories and the improvement of conditions, it seems that techniques based on whole genome sequencing, which have more discriminating power, will soon replace methods like PFGE."

**Keywords:** *Listeria monocytogenes*, molecular typing method, whole genome sequencing, MLST, PFGE

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Review: The anticancer effect of lactobacillus causes apoptosis induction in colorectal cancer cells

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

These days, Probiotics like lactobacillus have been recognized for their significant role in human health, especially the digestive system. These bacteria have numerous profits for preventing intestine inflammation, and diarrhea caused by bacteria and suppressing the proliferation of cancer cells that have a significant role in human health. In addition, lactobacillus can express different genes in colon cancer cells. In this review, the cytotoxic, anti-proliferative, and apoptotic effects of lactobacillus have been investigated on colon cancer cells.

#### MATERIALS AND METHODS

In this study, several articles and research articles on the use of lactobacillus and their effects on colon cancer, using databases such as Google Scholar, SID, NCBI, PubMed and Scencedirect were reviewed.

#### RESULTS AND DISCUSSION

Studies have shown using lactobacillus known as a probiotic can have effects on increasing expression of BAX (BCL-2), CASP3, and Beta-defensin 2 (BD-2) genes, and induces apoptosis in colon cancer cells Apoptosis induction in cancer cells results in various morphological changes that are indicators of apoptosis induction. It is worth noting that lactobacillus induce apoptosis in colon cancer cells, the cells stop during cell proliferation in the G0 and G1 phases of the cell cycle. Therefore, by reducing the proliferation of cancer cells, they play a significant role in preventing and treating colon cancer.

#### CONCLUSION

Probiotic has a significant impact on the improvement of the therapeutic process of colon cancer.

**Keywords:** Colorectal Cancer, Probiotic, Lactobacillus, BAX gene (BCL-2), CASP3 gene, Beta-defensin 2 (BD-2) gene

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Circulating *Brucella* spp. isolates in dairy cattle farms of Iran

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

Brucellosis is a widespread disease in developing countries like Iran. Cattle are mainly affected by *Brucella abortus*, although *Brucella melitensis* and *Brucella suis* can also sometimes cause infections in cattle. Common symptoms in female cattle include abortion and the retention of the fetal membrane, while males may exhibit signs of orchitis and bursitis. The transmission of brucellosis typically occurs through direct or indirect contact with infected cattle or their bodily fluids. This study aimed to determine the prevalence of brucellosis in cattle slaughtered in the Iranian slaughterhouses and identify the *Brucella* species circulating among these animals

#### MATERIALS AND METHODS

. A total of 525 cattle lymph node samples during 2021 and 2022 were collected from the different provinces of Iran and analysed by culture. Following the culture assay, all isolated bacteria were subjected to phage typing and AMOS PCR analysis.

#### RESULTS AND DISCUSSION

The prevalence of brucellosis in different locations was 19.4%, 12.4%, 0.9% and 0.3% for *Brucella melitensis* biovar 1, *Brucella abortus* biovar 3, *Brucella melitensis* biovar 3 and RB51 vaccine respectively. The 174 isolated samples were confirmed with PCR.

#### CONCLUSION

Implementing effective management techniques and raising public awareness about the transmission of brucellosis are crucial. Furthermore, there is a need for additional research on brucellosis in high-risk farms.

**Keywords:** *Brucella melitensis*, *Brucella abortus*, *Brucella suis*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluation of the efficacy of different concentrations of chitosan for biocontrol of pathogenic fungi on Citrus

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The annual report shows that a significant amount of fruit and vegetables are lost due to postharvest decay. Orange is one of the most popular citrus fruits. Molds are the main cause of its spoilage and reduce the quality of the products. Currently, synthetic fungicides are the first choice of treatment worldwide. Chitosan is known as a biological control agent due to its non-toxicity, biodegradability, and biocompatibility, and it's approved by the European Union RegEu20141563 for plant protection. This study was carried out to isolate and identify the main pathogenic fungi in oranges and to evaluate the inhibitory effect of different concentrations of chitosan as a biocontrol agent.

#### MATERIALS AND METHODS

Contaminated oranges were collected from the markets in Mohammadshahr City, Karaj, Iran. We isolated and selected five pathogenic fungi that were characterized macroscopically and microscopically by the slide culture method. The pathogenicity test was done in several stages to confirm the pathogenicity of the isolations. The isolated fungi were characterized based on genomic amplification and sequencing of the internal transcribed spacer region (ITS). In the next step, an aqueous chitosan solution with concentrations (2, 2.5, 3, and 3.5% w/v) is prepared by dissolving the specified amount in 1% acetic acid. After adjusting the pH to 5.6, the antimicrobial property of these solutions was tested against a suspension of 10<sup>6</sup> conidia/ml of each pathogenic fungus using the well diffusion test.

#### RESULTS AND DISCUSSION

The contaminated oranges were used for the isolation of pathogenic fungi, and these strains were tested for their ability to cause decay in healthy oranges. Five strains with the strong pathogenicity were characterized morphologically. Molecular identification indicates that the fungal strains belong to *Penicillium italicum*, *Penicillium raistrickii*, *Aspergillus ustus*, *Alternaria terricola*, and *Alternaria alternata*, respectively. The well diffusion test shows, the best concentration of chitosan was 3 percent (w/v), and we had the largest diameter of the zone of non-growth in this concentration.

#### CONCLUSION

This study suggests that a proper concentration of chitosan can be considered a potential biocontrol agent for the management of postharvest citrus diseases caused by some pathogenic fungi.

**Keywords: Orange Fruit, Citrus, Postharvest, Pathogen, Chitosan**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Pomegranate peel extract has antibacterial properties on MRSA

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

MRSA (Methicillin Resistance *Staphylococcus aureus*), as a multidrug-resistant bacterium, has been consistently one of the biggest concerns of patient treatment. Over the past years, the intrinsic properties of plant and fruit extracts have attracted scientists' attention to investigate. These properties, mostly, hint at antimicrobial activity toward pathogens which can eliminate the extended growth of these life-threatening microorganisms such as *Staphylococcus aureus*. This article aims to compare the effect of pomegranate juice and peel extract in different concentrations on MRSA and *S.aureus* bacteria to prove the existence of pomegranate's antimicrobial properties.

#### MATERIALS AND METHODS

The MRSA isolates were collected from the microbiology department of the Pasteur Institute of Iran. They were cultured aerobically on Muller Hinton Agar (MHA, Merck) and Muller Hinton Broth (MHB, Merck) mediums at 37 C for 24h. In this study, a juicer gathered fruit juice and then filtered it with 0/45 micrometer filters. Then, the pomegranate peel alcoholic extract, in certain concentrations (100- 600 mg/ml), was prepared using the soxhlet apparatus. For the investigation of antimicrobial activity, MIC and MBC methods were done. The micro-dilution and macro-dilution techniques were applied for pomegranate juice and peel extract.

#### RESULTS AND DISCUSSION

The MIC and MBC tests were applied for six concentrations of pomegranate peel alcoholic extract (100-600 mg/ml) and pomegranate juice on MRSA and *S. aureus*. Results showed that all concentrations of pomegranate peel alcoholic extracts had antibacterial activity and the MIC was 100 mg/ml, but the MIC and MBC tests for pomegranate juice on MRSA and *S. aureus* bacteria had no effective results. It can be illustrated that the pomegranate peel alcoholic extract can be more effective against pathogens. According to the studies done by Kaci FN, Ruzgar D, Gormez A, and Efe D in 2021, this research can be conducted.

#### CONCLUSION

The result of this study illustrates the strong antimicrobial activity of pomegranate peel extract on MRSA and *S.aureus* bacteria which can show us that pomegranate peel extract has more antimicrobial activity rather than pomegranate juice and for further research, it can be characterized as a useful supplement in industrial and pharmacological sciences to prevent the extended drug resistance that patients are suffering from.

**Keywords:** Pomegranate Juice and Peel Extract, Methicillin Drug Resistance, MRSA (Methicillin Resistance *Staphylococcus Aureus*)

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Yeast surface display: An updated insight into the recent applications and technology developments

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Yeast surface display has emerged as a powerful technology for the presentation of diverse biomolecules on the surface of yeast cells. In recent years, this versatile platform has witnessed significant advancements and found applications in various scientific disciplines.

### MATERIALS AND METHODS

PubMed, Web of Science, Scopus, and Google Scholar databases were used to study trends in yeast display and its applications. The review included approximately 50 relevant articles obtained using keywords like "yeast display" and "yeast surface display." We explore the utility of yeast surface display in protein engineering, antibody discovery, enzyme optimization, therapeutics development and biosensor construction. Moreover, we highlight the recent progress made in display methodologies, including the utilization of different yeast species, diverse display systems, and optimization strategies since 2019. The review also delves into emerging trends such as multi-gene display, library screening approaches, and the integration of synthetic biology tools with yeast surface display.

### RESULTS AND DISCUSSION

The presented overview emphasizes the growing significance of yeast surface display as a valuable platform for protein engineering and functional characterization. It underscores the potential for innovative applications in diverse research and industrial contexts, marking yeast surface display as a pivotal technology in the advancement of scientific knowledge and biotechnological development.

### CONCLUSION

This review provides an updated and comprehensive insight into the recent developments and applications of yeast surface display.

**Keywords:** Bacterial Autofluorescence, *Bacillus Subtilis*, Treatment Effect Investigation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and characterization of ESBL-producing *Klebsiella pneumoniae* isolates harboring $\beta$ -lactamase genes

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*K. pneumoniae* is a gram-negative, rod shape, non-motile, encapsulated bacteria. *K. pneumoniae* is one of the members of the enterobacteriaceae family that can cause a wide range of nosocomial infections. This bacteria considers a dangerous bacterium due to the high prevalence of antibiotic resistance, virulence factor genes, and extended-spectrum  $\beta$ -lactamase (ESBL) producing. Hence, the antibiotic choice to combat ESBL positive *K. pneumoniae* strains can be extremely limited. Thus, routine screening of ESBL-producing *K. pneumoniae* in health care settings can be a proper tool in order to decreasing ESBL producing strains emergence. This study aimed to determining *K. pneumoniae* isolates antibiotic resistance pattern, frequency of ESBL-producing isolates, and ESBL-related genes.

#### MATERIALS AND METHODS

In this study, clinical samples such as sputum, blood, and urine were used to isolation of desired bacteria. primary identification of isolates was performed by IMViC tests, then, amplification of specific 16s rRNA by PCR method was used as final identification of isolates. The antibiotic resistance pattern was determined by disk diffusion method via ampicillin (10  $\mu$ g), aztreonam (30  $\mu$ g), amikacin (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), tetracycline (30  $\mu$ g), ceftriaxone (30  $\mu$ g), ceftazidime (30  $\mu$ g), gentamicin (10  $\mu$ g), imipenem (10  $\mu$ g), and trimethoprim-sulfamethoxazole (1.25/23.75  $\mu$ g) disks according to CLSI guidelines. Evaluation of ESBL production was performed by the double disk synergy test (DDST) method. In the end, the prevalence of  $\beta$ -lactam genes including blaNDM-1, blaSHV, blaOXA-48, and blaOXA-23 in ESBL-producing isolates were investigated by PCR technique.

#### RESULTS AND DISCUSSION

In this study, 50 different *K. pneumoniae* isolates (58% males, 42% females) were identified. Compatible with other researches, the isolates of this bacterium showed high antibiotic resistance in this study. The isolates were most resistant to ampicillin (98%), following tetracycline (76%), amikacin (58%), ceftriaxone (50%), ciprofloxacin (50%), gentamicin (38%), trimethoprim-sulfamethoxazole (30%), imipenem (20%), and aztreonam (10%). Every isolate was resistant to at least one group of antibiotic classes that were used in the present study, moreover, the lowest resistance was against the antibiotic aztreonam, while the highest resistance was found against ampicillin, this is compatible with the fact that *K. pneumoniae* strains are resistant to ampicillin intrinsically due to presence of blaSHV-1 genes in this bacteria. In addition, the frequency of ESBL-positive isolates was determined as 18%. Frequency of blaNDM-1, blaSHV, blaOXA-48, and blaOXA-23 genes among ESBL-producing isolates were 55.55%, 44.44%, 33.33%, 77.77% respectively. According to other studies conducted in Iran, ESBL-producing *K. pneumoniae* isolates specially NDM-1 and OXA-48 harboring isolates have been increasing in the past several years, however, the frequency of these isolates in the present study was less than mentioned papers even with the study that were conducted in Tabriz city. It can be due to the factors like geographical diversity, location of study, population density, administering antibiotics, and personal hygiene, it seems investigation of the mentioned factors can be very helpful in understanding this phenomenon.

#### CONCLUSION

In conclusion, in this study  $\beta$ -lactamase harboring ESBL-positive *K. pneumoniae* isolates were identified, however, large-scale research including the characterization community and nosocomial infections-related *Klebsiella* spp. strains from different regions of Iran are required to offer information on the spread of these microorganisms in this country.

**Keywords:** Antimicrobial resistance, *K. pneumoniae*, ESBL

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular characterization of ESBL-producing *Escherichia coli* isolates from ICU patients in Tabriz - Iran

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

*E. coli* is a gram-negative, rod-shaped bacterium and a member of the Enterobacteriaceae bacterial family that is considered an opportunistic bacterial pathogen with high pathogenic features. Also, antibiotic resistance and relative issues, such as Extended-spectrum  $\beta$ -lactamase (ESBL) production, have risen in *E. coli* in recent decades. The molecular screening of antibiotic resistance can give physicians an excellent perspective to choose the right therapeutic agent and help scientists design alternative therapeutic approaches. Therefore, this study was conducted to determine the prevalence of ESBL production-related genes, such as blaOXA-23, blaOXA-48, blaSHV, and blaNDM-1.

### MATERIALS AND METHODS

50 *E. coli* isolates were examined in this study. Isolates identification is performed by biochemical (IMViC tests) and molecular (amplifying 16s rRNA gene) methods. Antibiotic resistance pattern evaluated by disk diffusion method via 10 different antibiotic disks including ceftazidime (30  $\mu$ g), imipenem (10  $\mu$ g), gentamicin (10  $\mu$ g), trimethoprim-sulfamethoxazole (1.25/23.75  $\mu$ g), ceftriaxone (30  $\mu$ g), tetracycline (30  $\mu$ g), aztreonam (30  $\mu$ g), ampicillin (10  $\mu$ g), amikacin (30  $\mu$ g), and ciprofloxacin (5  $\mu$ g) according to CLSI guidelines. Double disk synergy test (DDST) was performed for morphological determination of ESBL-producing isolates. In addition, the frequency of 4 ESBL-related resistance genes, including bla<sub>OXA-23</sub>, bla<sub>OXA-48</sub>, bla<sub>SHV</sub>, and bla<sub>NDM-1</sub>, was determined by PCR in ESBL-producing isolates.

### RESULTS AND DISCUSSION

Of the 50 isolates examined in this study, 48% were isolated from men and 52% from women. According to previous studies conducted in Iran, this bacterium seems problematic in Iranian women. Since this bacterium causes disorders such as preterm birth (PTB) and asymptomatic bacteriuria (ABU) during the pregnancy period. These disorders can result in abortion, early delivery of the fetus, and also financial loss. It seems Screening and diagnostic tests for this bacterium in pregnant women seem necessary. In this study, all isolates were resistant to at least one of the antibiotic groups. Also, ampicillin has the highest resistance rate among other antibiotics. The prevalence importance of ampicillin-resistant *E. coli* strains in patients has been highly investigated in previous studies. This resistance occurred due to indiscriminate administration and the availability of ampicillin in the market. Consequently, many *E. coli* strains have developed  $\beta$ -lactamases encoded by SHV and TEM genes in order to inactivate ampicillin. Thus, the prescription and use of ampicillin to treat this bacterium should be limited. 24% of *E. coli* strains were determined as ESBL producers. Furthermore, in the current study, ESBL-related genes, including OXA-48, OXA-23, NDM-1, and SHV frequency, were 8.33%, 58.33%, 33.33%, and 50% respectively. Comparing the results of this study with a meta-analysis that was conducted recently indicated that the SHV gene is increased in the past few years among Iranian patients. Also, the frequency of the NDM-1 gene in this study is higher than in similar studies conducted in Pakistan and Taiwan. Therefore, it seems the presence of NDM-1 in *E. coli* strains in Iran can be problematic.

### CONCLUSION

In summary, *E. coli* isolates with high antibiotic resistance patterns harboring ESBL-producing relative genes were identified in this study. However, the lack of molecular pathotyping of *E. coli* isolates can be a major limitation of the present study. Like what was done in the present study, routine screening of ESBLs, to prevent the spreading of ESBL-producing isolates in hospital environments was suggested.

**Keywords:** Antimicrobial resistance, *E. coli*, ESBLs

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# Culture-dependent comparison of gastric microbiota in patients with chronic gastritis and gastric atrophy

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Chronic gastritis and gastric atrophy, which is characterized by the loss of normal gastric mucosal cells, are two common stages of gastric diseases. The role of gastric microbiota in the pathogenesis of these diseases is still under investigation. In this primary study, the composition of culturable gastric microbiota was compared in patients with chronic gastritis and atrophy using conventional phenotypic methods.

### MATERIALS AND METHODS

Gastric biopsy samples were obtained from 5 patients classified in gastritis and 5 patients in atrophy groups by pathological survey. After homogenization and initial enrichment, serial dilutions of each biopsy were inoculated on different selective and non-selective media. Incubation was done in two temperature conditions (28°C and 37°C), both aerobically and anaerobically. After 24-72 hours, the grown colonies were examined by microscopic and macroscopic methods.

### RESULTS AND DISCUSSION

Totally 28 distinct bacterial colonies were identified. Most of the bacterial strains were G<sup>+</sup> bacilli which were isolated under anaerobic condition. 13 isolates (11 G<sup>+</sup> and 2 G<sup>-</sup>) were obtained from patients with chronic gastritis and 15 isolates (13 G<sup>+</sup> and 2 G<sup>-</sup>) from atrophic patients. Out of 13 isolates from patients with chronic gastritis, 8 strains were bacilli and 5 strains were cocci form. Out of 15 isolates from atrophic patients, 9 isolates were bacilli and 6 isolates were cocci forms.

### CONCLUSION

Recent studies showed that microbiota of the stomach may also influence the development of gastric disorders. In this pilot study, morphologically-different bacteria were isolated from gastritis and atrophic patients. PCR-based identification of isolated bacteria targeting prokaryotic universal *16S rRNA* gene will give more clear insight into gastric bacterial populations in these two patients group and their impact on gastric diseases.

**Keywords:** Gastric atrophy, Gastritis, Microbiota

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# Isolation and host range analysis of bacteriophages against XDR Extraintestinal Pathogenic *Escherichia coli* isolates from broiler chickens with Colibacillosis

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Colibacillosis is an infectious disease that has a significant economic impact on poultry breeders worldwide. This contagious illness is brought on by the specific extra-intestinal pathogenic *E. coli* (ExPEC) known as avian pathogenic *E. coli* (APEC). Due to its zoonotic potential and high antibiotic resistance pattern, this bacteria is one of the most troublesome bacterial species. This research aimed to identify bacteriophages specific to multidrug-resistant (MDR) and extensively drug-resistant (XDR) APEC isolates and evaluate the lysis capability of isolated phages.

### MATERIALS AND METHODS

In this study, 196 samples were collected from poultry with Colibacillosis and chicken farms in East Azerbaijan province between June and September 2022. One hundred different APEC isolates were obtained from feces samples, internal organs of chicken carcasses, and food samples. Biochemical tests were performed for initial identification, and then final confirmation was performed by 16s rRNA, iucD, papC, and fimC gene amplifications. Antibiotic susceptibility test evaluated by disc diffusion method. 5 XDR and 5 MDR isolates were used as hosts for bacteriophage isolation. Ssewage water samples were collected from Maragheh and Tabriz City, East Azerbaijan, Iran. Phage isolation was performed by Pallavali et al. method. Then, the Adams soft agar method was used to detect of desired phages. Also, phage purification was performed by Sambrook et al. method (with 3-time repetition). Finally, the host range of purified phages was determined by spot test.

### RESULTS AND DISCUSSION

Antibiotic susceptibility tests indicated that 5 strains were XDR, 87 were MDR, and 8 could not classify into any drug resistance relative groups. The Prevalence frequency of papC, iucD, and fimC virulence factors were 17%, 66%, and 77% respectively. Most APEC strains use fimC, and iucD products, as virulence factors. Thus, fimC and iucD products can be suitable

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for vaccine and anti-virulence targets. Totally 20 different phages were isolated, and the host range of phages was between 4% (effective on only 1 strain) and 56% (effective on 14 strains). The host range of phages is one of the essential characteristics of these viruses to be chosen as a potential therapeutic agent. However, there is no comprehensive definition for broad-spectrum phages until now. Therefore, viruses with a broader spectrum are usually selected for further experiments in similar studies. Our study determined the phage with lytic activity against 56% of strains as a broad spectrum. In contrast, in similar studies, phages with a spectrum of 60%, 40%, and even 5% have been selected as broad-spectrum bacteriophages.

### CONCLUSION

In summary, the high frequency of MDR drug resistance in APEC isolates with the highest resistance to tetracycline, nalidixic acid, and doxycycline was founded in this study. Additionally, lytic bacteriophages were isolated, and the spectrum of each phage was determined. However, further studies are needed to investigate the presence of different microbial resistance genes and other virulence factors in APEC isolates. It is also suggested to continue sampling wastewater to obtain bacteriophages with a broader spectrum. Overall, bacteriophages can be used to combat antibiotic-resistant bacteria. However, further studies are necessary to quantify the spectrum of the isolated phages and evaluate other characteristics of these phages.

**Keywords:** Antimicrobial resistance, Extra-intestinal pathogenic *E. coli*, Bacteriophage, Phage therapy

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# Phenotypic survey of cultivable gastric bacteria obtained from patients with gastritis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Gastritis, characterized by inflammation of the stomach lining, is a common gastric disorder with various causes. Recent studies showed that microbiota of the stomach may be one of the important etiologic factors for development of various gastric diseases. In this study, gastric bacteria of five gastritis patients were isolated and initially assessed by conventional macroscopic and microscopic methods.

### MATERIALS AND METHODS

Five gastric biopsies were obtained from gastritis patients. After homogenization and enrichment, different dilutions of each biopsy were prepared and inoculated on MRS, Brain Heart Infusion blood agar and Mueller Hinton agar under anaerobic (using glove box) and aerobic conditions. Incubation was performed at 28°C and 37°C both aerobically and anaerobically. Cultures were examined after 48-72 hours for colony characteristics and bacterial morphology.

### RESULTS AND DISCUSSION

At least one distinct colony was isolated from each patient. Totally, 13 bacterial morphology forms were identified; five G<sup>+</sup> bacilli, four G<sup>+</sup> cocci, two G<sup>+</sup> coccobacilli, one G<sup>-</sup> coccobacilli and one G<sup>-</sup> bacilli. Based on incubation condition, 9/13 strains were isolated anaerobically and 4/13 aerobically. Furthermore, 9/13 isolates observed at 37°C and 4/13 at 28°C.

### CONCLUSION

Analysis of culturable gastric bacteria obtained from patients with gastritis provided primary valuable insights into their morphological characteristics. Further studies using molecular techniques on these patients as well as other dyspeptic patients could elucidate more details about composition of gastric microbiota and its clinical significance. Furthermore, some isolated bacterial species may provide health benefits and therefore could represent future candidates for microbiota modulating therapies.

**Keywords:** Gastric microbiota, Gastritis, morphological characteristics

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# Investigation of resistance to fluoroquinolones among *Klebsiella pneumoniae* isolates recovered from patients referred to hospitals affiliated to Ardabil University of Medical Sciences, 2020-2021

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Klebsiella pneumoniae* is one of the important causes of nosocomial infection and leading causes of ventilator-associated pneumonia. Apart from pneumonia, this bacterium can cause a wide range of infections such as wound infection, cholecystitis, meningitis, septicemia, osteomyelitis, diarrhea, and urinary infection. Antibiotic resistance is one of the most important challenges in treating infections caused by bacteria, such as *Klebsiella pneumoniae*. This study was designed to investigate the antibiotic resistance of clinical isolates of *Klebsiella pneumoniae* to fluoroquinolones used to treat the disease in Ardabil hospitals.

### MATERIALS AND METHODS

In this descriptive, cross-sectional study, 100 isolates of *Klebsiella pneumoniae* were recovered from patients referred to hospitals affiliated to Ardabil University of Medical Sciences between 2020-2021. Biochemical and molecular methods were used to identification of the isolates. The antibiotic resistance pattern was done using disc diffusion method based on CLSI guidelines. The minimum inhibitory concentration (MIC) of ciprofloxacin was determined by agar dilution method and mutations in *gyrA* and *parC* genes and the presence of resistance genes such as *accA*, *qnr*, and *qepA* were investigated using PCR method.

### RESULTS AND DISCUSSION

In this study, 49% of the isolates were MDR strains, and the highest rate of resistance to cefazolin (66%) and cefotaxime (66%) was observed. Meropenem and amikacin with 76% and 69% sensitivity were the most effective antimicrobial agents against *Klebsiella pneumoniae*. *accA* resistance gene was detected in 10 isolates (10%), while *qepA* and *qnr* resistance genes were not observed in any of the isolates. The MIC range of ciprofloxacin was between 0.5 and above 1024, with 4 isolates having a MIC above 1024. These isolates were examined for mutations in *gyrA* and *parC* genes. In the investigation of mutation in *gyrA*, an amino acid substitution from serine to isoleucine was observed at position 83, while no mutation was observed in *parC* gene.

### CONCLUSION

Ciprofloxacin is an effective drug against complicated urinary infections, pyelonephritis, wound infection, and respiratory infections. In this study, a high frequency of *aac (6)-Ib-cr* gene was reported. Since this gene is located on a plasmid, it can easily transfer between *Klebsiella pneumoniae* strains in hospital settings, leading to the spread of resistance to fluoroquinolones.

**Keywords:** *Klebsiella pneumoniae*, Meningitis, Septicemia, Antimicrobial resistance, Fluoroquinolones

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Assessment of Antibiotic Resistance Genes in *Escherichia coli* Isolates from Hospital Environments

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Antibiotic resistance increases the prolonged consequences and mortality caused by *E. coli* infections. The emergence and expression of resistant genes is a potential factor for the stability of this pathogen against antibiotics. Hence, the present study was designed to investigate the prevalence of antibiotic resistance genes in *E. coli* isolates from Tabriz, Iran.

### MATERIALS AND METHODS

In this study, 141 females and 78 males aged 1 to 89 from surgery, internal, intensive care unit, and pediatric wards of Tabriz hospitals were enrolled. *E. coli* was isolated from 219 biological samples (urine, blood, wounds, peritoneum, and respiratory tracts) by culture on sheep blood agar or MacConkey agar and microbial detection standards. The genomic DNA was extracted using cetyltrimethylammonium bromide and resistance genes were identified by polymerase chain reaction method.

### RESULTS AND DISCUSSION

In the molecular investigation, at least 4 high risk genes inducing resistant for *E. coli* isolates were reported in each antibiotic group. The interpretation of data for each of the antibiotic groups indicates that *bla*<sub>CTXM-15</sub> (70%) among positive beta-lactamases, *tet*<sub>B</sub> (31.6%) in tetracycline, *fos*<sub>C</sub> (40%) in fosfomycin, *Oq*<sub>XB</sub> (34%) in fluoroquinolone, *Arm*<sub>A</sub> (12.96%) in aminoglycosides, and *Sul*<sub>I</sub> (69.5%) in co-trimoxazole combination are genetic indexes of antibiotic resistance.

### CONCLUSION

The genes encoding the destructive factors of antibiotics had a significant prevalence in *E. coli* isolates. The *bla*<sub>CTXM-15</sub> inducing beta-lactam resistance was the most common gene among our clinical isolates. Due to the possibility of development of resistance genes with chromosomal or plasmid origin, the consumption of antibiotics based on approved global standards has a high priority.

**Keywords:** *E. coli*, antibiotic resistant genes, PCR, Azerbaijan, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of Antimicrobial Efficacy in *Escherichia coli* Isolates from Hospital Settings

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Escherichia coli* (*E. coli*) as a gut commensal bacteria, is a common candidate for most gram-negative infections of humans. Unplanned administration of antibiotics to control or treat *E. coli* infection leads to the activation of microbial resistance mechanisms and insufficient effect of therapeutic antibiotics. This epidemiological study was conducted to investigate the sensitivity of a wide range of antibiotics against *E. coli* isolates from Tabriz, Iran.

### MATERIALS AND METHODS

From July 2019 to June 2020, 219 samples of urine, blood, wound, respiratory tract, and peritonea were collected from different hospital wards such as surgery, internal, intensive care unit, and pediatrics. *E. coli* isolates were obtained by culture on sheep blood agar and MacConkey agar and microbial detection tests. To determine the antibiotic sensitivity or resistance of the isolates, disk diffusion agar and minimum inhibitory concentration tests were selected.

### RESULTS AND DISCUSSION

*E. coli* isolates were the most resistant to ampicillin (99%) and the least resistant to imipenem and fosfomycin (3%). The trend of the highest antibiotic resistance continued with sulfamethoxazole (87%) and trimethoprim (78%). The resistance of *E. coli* isolates to the combination of these two antibiotics in the form of co-trimoxazole was also a high percentage (70%). The isolates from the internal and then surgical wards were high resistance. There was no significant correlation between age or gender and resistance of *E. coli* isolates.

### CONCLUSION

This study indicated that *E. coli* has become a potential pathogen in Eastern Azerbaijan, Iran. *E. coli* isolates were remarkably resistant to a large number of antibiotics. Antimicrobial resistance prevalence was associated with hospital wards. If consumed wisely, fosfomycin is the current appropriate choice for controlling *E. coli* infection.

**Keywords:** *Escherichia coli*, Drug Resistance, Antibiotics, Tabriz, Iran

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# Investigation of the Prevalence of Anti-*Helicobacter pylori* Antibodies in the Azerbaijani Population of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Helicobacter pylori* (*H. pylori*) is a widespread pathogenic agent that often invades gastric mucosa epithelial cells. Invasion of this dominant bacterial species of stomach can induce mucosal cellular immunity and local and systemic humoral immunity. Hence, it is possible to identify common *H. pylori* infection by assessing host immune responses as a non-invasive method. In this research we aimed to detect the abundance of serum immunoglobulin (Ig) G, IgM and IgA and their association with the age and gender of participants from children to adults in Tabriz, Iran.

### MATERIALS AND METHODS

Between 2019-2022, venous serum samples of 3733 normal participants (1235 males, 2498 females) were collected for immunodiagnosis of *H. pylori* infection. The enzyme-linked immunosorbent assay method was used to detect the antibody groups. The statistical data was analyzed by SPSS software.

### RESULTS AND DISCUSSION

According to comparison results, the prevalence of IgG in 1949 individuals (57.9%), IgA in 83 individuals (11.6%), and IgM in 8 (0.3%) participants were reported positive. In terms of seropositivity, females were the significant target group for only IgM. While for both IgA and IgG, the number of seropositive males was predominant with a non-significant difference. Moreover, a significant correlation was observed between ageing and positive prevalence of IgG, IgM and IgA antibodies in biosamples. In some age subgroups, gender was also a remarkable factor for evaluating the prevalence of serum indicators such as IgG and IgM.

### CONCLUSION

Similar to most cities in Iran, there is an unstable situation in the capital of East Azerbaijan province against *H. pylori* infection. Out of three immunoglobulin groups, IgG had the highest serum prevalence among our study population. Also, adult age groups were more at risk of *H. pylori* infection. Due to the growing prevalence of this pathogen, it is necessary to pay attention to infection control programs of developed countries and promoting basic health education.

**Keywords:** anti-*Helicobacter pylori* antibodies, serological assay, stomach inflammation, Tabriz

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# Postbiotics: new generation of probiotics and safe alternative in food and pharmaceutical industries

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Since probiotics promote digestive health and regulate microorganisms, they have become widely available to the public in the form of supplements and probiotic products. Despite their widespread use, concerns exist regarding the potential side effects of consuming live bacteria, especially in individuals with underlying conditions. The identification of certain non-living metabolites derived from probiotics, known as postbiotics, has revolutionized the food and pharmaceutical industries. This review study examines postbiotics and their health-promoting effects in the food and pharmaceutical industries.

### MATERIALS AND METHODS

In this study, several articles and research papers on the application of postbiotics in the food and pharmaceutical industries were reviewed using databases such as Google Scholar, Scopus, and SID.

### RESULTS AND DISCUSSION

In a study measuring the amount of probiotics and postbiotics in a probiotic food product, it was found that the number of non-living cells exceeded the number of live cells. Consequently, it was determined that the health effects of probiotic products are more influenced by postbiotics. Another study conducted on a group of children aged 12 to 48 months showed that daily consumption of postbiotics derived from *Lactobacillus paracasei* led to a reduction in the occurrence of diarrhea, acute gastroenteritis, and pharyngitis inflammation. In another study, it was observed that propionate derived from *Propionibacterium freudenreichii* increased apoptosis in cancer cells. Postbiotics derived from *Lactobacillus* were found to inhibit colon cancer. Clinical evidence obtained in a study showed that postbiotics, including butyrate, play a significant role in reducing food allergies. Furthermore, a study conducted on children with lactose intolerance revealed a significant correlation between the reduction of symptoms and the consumption of postbiotics.

### CONCLUSION

Postbiotics can be a good alternative in the treatment and prevention of diseases and the production of beneficial foods. Compared to probiotics, they are more cost-effective, have better stability, and are clinically safer. The identification of new postbiotics and their incorporation into industries in a way that preserves their health-promoting properties, as well as determining the appropriate dosage in pharmaceutical supplements, requires further extensive research.

**Keywords:** Postbiotics, Beneficial Foods, Probiotics, Biological Activity, Health Effect

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### The inhibitory effect of gB, gH/gL antibodies on the spread of HSV

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Human herpes virus is one of the most common viruses in humans that cause latent or overt infections. On the other hand, the emergence of drug resistance necessitates the development of new antiviral drugs. Glycoproteins are the basis of the entry and integration of the virus into the host cell. Blocking this step can prevent the virus from attaching and entering the host cell, so that any defect in the glycoprotein complex prevents entry and fusion. gD is responsible for binding to the host cell. After gD, gB and gL/gH bind to their receptors, they cause fusion and entry of the virus into the host cell and cause disease. The purpose of this study is to investigate the inhibition of gB and gL/gH and finally the inhibition of virus fusion and entry with antibody design.

#### MATERIALS AND METHODS

Using the UniProt database and obtaining information about HSV virus glycoproteins and their cellular receptors, we retrieved them. With SWISS-MODEL, we modeled the cell receptor as an antibody and in the last step, glycoproteins and antibodies were docking using clus pro.

#### RESULTS AND DISCUSSION

Based on the results of bioinformatics analysis, the mentioned structure has a high binding power to the virus and can prevent the fusion of the virus to human host cells.

#### CONCLUSION

This designed structure can successfully bind to its viral receptor and inhibit the binding site of the virus and prevent the virus from entering the cells. This antibody has the potential to be developed into a suitable vaccine or drug against HSV, although laboratory studies and clinical trials must be performed.

**Keywords:** Human herpes virus, Glycoproteins, HSV, gB , gH/gL

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# The effect of gold nanoparticles on ciprofloxacin-resistant *Staphylococcus aureus* isolates from skin infections in Qom province

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

In the development of multidrug resistance, efflux pumps effectively pump drug compounds out of cells, which results in reduced membrane permeability to drug compounds. This study evaluated the effect of gold nanoparticles on ciprofloxacin-resistant *Staphylococcus aureus* isolated from burn patients in Qom province, Iran.

### MATERIALS AND METHODS

To this end, 88 *Staphylococcus aureus* strains from skin infection samples in Qom province were identified in genotypic and phenotypic manner using different biochemical methods. The antimicrobial effects of the compounds were measured by disc diffusion and MIC methods according to the CLSI protocol.

### RESULTS AND DISCUSSION

Of 88 *S. aureus* strains tested, 50 (56.81%) were resistant to ciprofloxacin by disc diffusion method and MIC microdilution was done on them. The highest concentration of inhibitor in 20 strains (40%) was obtained at 128 µg / ml during microdilution. Gold nanoparticles alone did not have an effect on *Staphylococcus aureus* growth inhibition, but in microdilution with ciprofloxacin in the presence of nanoparticles, the minimum growth inhibition concentration of strains was half that of Nano-free MIC.

### CONCLUSION

Gold nanoparticles with ciprofloxacin could be used to prevent the expression of pump genes involved in resistance to fluoroquinolone compounds.

**Keywords:** *Staphylococcus Aureus*; Ciprofloxacin; Gold Nanoparticles; Minimum Inhibitory Concentration (MIC); Multidrug Resistance.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Inhibitory effects of cell extract from *Lactobacillus* species on K562 cell lines

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Leukemia, a malignancy affecting the hematopoietic cells, has a high mortality rate in individuals under 20 years old. Resistance to chemotherapy has led to the expansion of research based on alternative treatment. Studies indicated that certain bacterial proteins and short peptides possess inhibitory properties against various tumor cells. The purpose of a literature review is to research on the beneficial effects of probiotics on the K562 cell line, which consists of myeloid-derived blood cancer cells.

### MATERIALS AND METHODS

This review, analyze the studies conducted between 2011 and 2023, focusing the impact of various types of *Lactobacillus* species on the K562 cell line. scientific databases such as Google Scholar, Scopus, PubMed, and SID were selected for this review.

### RESULTS AND CONCLUSION

The results of this review indicated that the cell wall and cytoplasmic extract of *Lactobacillus casei*, *paracasei*, and *reuteri* have significant cytotoxicity against K562 myeloid cancer cells. The cell wall protein fractions derived from different *Lactobacillus* strains inhibit K562 cancer cell growth and protein fractions with higher molecular weight notably exhibit greater anticancer effects. The survival rate of cancer cells was related to the concentration and duration of exposure to lactobacilli-derived cell extracts. Moreover, the specific beneficial effects of these bacteria in inhibiting cancer cell growth may be vary between different *Lactobacillus* species. The mutated p53 gene, (tumor suppressor), contribute to cancer development. Heightened expression of mutated p53 proteins enhances resistance to apoptosis. Thereby, the cytoplasmic extract and cell wall of *Lactobacillus* induce apoptosis in K562 cells by reducing mutated p53 protein levels.

The extract obtained from *Lactobacillus* bacteria can be utilized as a component of a pharmaceutical formulation for the effective treatment of myeloid leukemia.

**Keywords:** K562 Cell Line, *Lactobacillus* Species, Cancer Therapy

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the antibiotic resistance pattern of *Staphylococcus aureus* isolated from clinical samples

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Staphylococcus aureus* is the cause of many infections. after observing resistance to penicillin, methicillin was used for treatment. Gradually, strains resistant to methicillin (MRSA) were created, glycopeptide antibiotics such as vancomycin are one of the most effective antibiotics for the treatment of these strains, but due to the observation of strains with intermediate resistance to vancomycin, the use of these antibiotics is limited and very controlled.

### MATERIALS AND METHODS

The identification of *Staphylococcus aureus* present in suspected clinical samples was done with the help of phenotypic method which is based on biochemical tests .

After identifying bacteria, phenotypic resistance detection method was used to identify resistant bacteria..

### RESULTS AND DISCUSSION

The results of determining the antibiotic sensitivity of *Staphylococcus aureus* by disc diffusion method for the studied antibiotics showed: the bacteria had the highest resistance to the antibiotic penicillin first (66.6%) and then tetracycline (49.9%), the most effective antibiotic was gentamicin (100% sensitivity of the strains), the most interstitial resistance was also observed to the antibiotic vancomycin (41. 6%)

### CONCLUSION

Examining the sensitivity of strains isolated from patients, in addition to determining the most suitable antibiotic for treatment, leads to the timely identification of strains that have intermediate resistance to some antibiotics to which complete resistance is rarely seen, such as vancomycin. Awareness of the progression of resistance among strains leads to the timely presentation of efficient approaches to prevent and treat related diseases and control the spread of resistant strains in the population.

**Keywords:** *Staphylococcus Aureus*, Antibiotic Resistance, Methicillin, Vancomycin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Advanced Archaea as Potential Causes of Unknown Diseases

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Archaea, a distinct group of microorganisms that thrive in extreme environments, have long captured the fascination of scientists and researchers. This article explores the potential connection between advanced archaea and unknown diseases, shedding light on the fascinating and uncharted world of these microorganisms.

#### Advanced Archaea and Disease:

While many archaea are harmless and play important roles in the ecosystem, recent studies have highlighted the presence of advanced archaea with novel characteristics that may be linked to unknown diseases. These archaea possess unique genetic and metabolic capabilities, making them potential sources of novel infectious agents.

#### Potential Mechanisms of Archaea-Related Diseases:

Understanding the mechanisms by which advanced archaea may cause disease is crucial in unraveling their impact. While this area of research is in its infancy, several theories have emerged. One possibility is that advanced archaea may directly infect cells, causing tissue damage and triggering an immune response. Another hypothesis is that advanced archaea may produce toxins or metabolic byproducts that have detrimental effects on host cells.

#### Challenges and Future Directions:

Studying advanced archaea and their potential role in disease poses several challenges. These microorganisms are difficult to culture and study in the laboratory. Moreover, our understanding of archaeal genetics and metabolism is still limited, which hinders our ability to fully comprehend their impact on human health.

## CONCLUSION

this uncharted microbial world offers an exciting avenue for research and may provide valuable insights into the prevention, diagnosis, and treatment of these enigmatic diseases. Continued scientific investigation will unlock the mysteries of advanced archaea, leading the way to a better understanding of their impact on human health.

**Keywords:** Archaea, Potential, Disease

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Simultaneous CO<sub>2</sub> Bio-fixation and Wastewater Treatment by Microalgae

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Using photosynthetic microorganisms for carbon dioxide (CO<sub>2</sub>) bio-fixation is one of the ways to prevent releasing it into nature and reduce the effects of greenhouse gases and global warming. Microalgae, found in freshwater and saltwater, are capable of producing oxygen and biomass with sunlight, water and CO<sub>2</sub>. The produced biomass can eventually be used for food, medicine, cosmetics and as source of biofuel. Ayatollahi et al. (2021) prepared experiments with five CO<sub>2</sub> concentrations (air-5-10-15-20 %), synthetic municipal wastewater and *C.vulgaris* cultivation on it. The results revealed that the best growth rate of microalgae achieved at 5% of CO<sub>2</sub> concentration with a C/N ratio of 4, of the wastewater.

### MATERIALS AND METHODS

In this research, *Chlorella vulgaris* microalgae was used for CO<sub>2</sub> bio-fixation. In order to reduce costs and also save water consumption, the wastewater of the Tuna processing factory was used as a cultivation medium. Three concentrations of CO<sub>2</sub> (5%-10%-15% in N<sub>2</sub>) at different dilutions of wastewater in distilled water (1:4, 2:4, 3:4 and 4:4) were investigated to determine the best conditions for *C. vulgaris* growth. Cultivation was carried out at room temperature. Lighting was cyclic (12-12 h), at intensity of 6000 lux. Cultivation was done in two systems, batch (serum bottles) and semi-continuous (airlift photobioreactor). In both systems, wastewater analyzed at the beginning and the end of the experiments to determine the COD, total phosphorus, nitrate and ammonium removals. CO<sub>2</sub> consumption also detected by GC to give an insight into the simultaneous gas consumption, biomass production and wastewater treatment.

### RESULTS AND DISCUSSION

The results showed microalgae's ability to grow in all concentrations of CO<sub>2</sub> in both systems. In semi-continuous photobioreactor after 23 h, (at 5% gas) the CO<sub>2</sub> stabilization and the effluent analysis of 1:4 dilution, both showed more than 50% of gas bio-fixation and nitrate removal, respectively.

### CONCLUSION

The results were promising showing the ability of the system for simultaneous CO<sub>2</sub> bio-fixation and wastewater treatment.

**Keywords:** Microalgae, CO<sub>2</sub> Bio-fixation, Tuna processing wastewater.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of Vancomycin-Resistant Enterococci and antibiotic resistance pattern in patients referred to Ganjavian hospital, Dezful.

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nowadays, the resistance of bacteria to antibiotics has affected the success of treatment of infectious diseases all over the world. In recent decades, the role of enterococci as one of the causative factors of hospital infections in urinary infections, wounds and fatal bacteremia has become very important. Severe complications of bacteremia and enterococcal endocarditis are associated with high mortality.

### MATERIALS AND METHODS

In this cross-sectional study (July 2022 to July 2023), clinical samples of blood, urine, wounds and body fluids of outpatients and inpatients at Ganjavian hospital were collected and cultured on Blood and MacConkey agar. The phenotypic identification of the isolates was done by gram staining and biochemical tests of catalase, arabinose fermentation and bile esculin hydrolysis, and antibiotic sensitivity testing was done by disc diffusion method based on CLSI. The resistance of enterococcus strains to vancomycin was determined by the vancomycin agar method. Genotypic identification of isolates was done by PCR method of *ddl* and *vanA* genes.

### RESULTS AND DISCUSSION

In the present study, 120 enterococcus including *Enterococcus faecium* and *E. faecalis* were collected with frequency of 60.7% and 39.3%, respectively, which were collected from patients of internal departments with 31.4%, ICU with 20.9%, and then outpatients. Most of the samples included urine culture 76.6%, wound culture 10.4% and blood culture 7.8%. In vancomycin agar method (32.5%) 39 resistant isolates were identified, of which 89.7% were *Enterococcus faecium*. *vanA* gene was detected in 21 isolates. The antibiotic resistance of VRE isolates to penicillin, ampicillin, ciprofloxacin, and tetracycline was observed as 87.2, 87.2, 94.9, and 76.9%, respectively, and nitrofurantoin was the most effective antibiotic in urinary infections.

### CONCLUSION

The resistance of one third of enterococci to vancomycin, which is one of the most widely used antibiotics in the treatment of gram-positive infections, is a serious concern in the treatment of infectious patients, which requires more monitoring by infection control committees and the establishment of the antimicrobial stewardship program and has access to newer and more effective antibiotics.

**Keywords:** Vancomycin-resistant enterococci, Antibiotic resistance, *ddl* and *vanA* genes, PCR.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Phenotypic and genotypic evaluation of extended spectrum $\beta$ -lactamase-producing *Escherichia coli* isolated from bovine clinical mastitis cases and Fecal strains, Mashhad, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Extended-spectrum  $\beta$ -lactamase (ESBL)-producing Enterobacteriaceae have become a serious public health hazard worldwide. This study aimed to identify and investigate the occurrence of ESBL-producing *Escherichia coli* strains, isolated from bovine clinical mastitis cases and Non-pathogenic bovine fecal strains in Mashhad Province.

### MATERIALS AND METHODS

A total of 107 isolates were studied, 75 *E. coli* strains were isolated from bovine clinical mastitis cases, and 32 isolates were non-pathogenic fecal strains. To phenotypic confirmation of ESBL-producing strains, combination disc diffusion method was applied according to the CLSI guidelines. Additionally, Genotypic and sequencing analyses of ESBL resistance genes were conducted on all isolates by using polymerase chain reaction.

### RESULTS AND DISCUSSION

Overall, 12 of 107 *E. coli* isolates (11.21%) were identified as ESBL-producers. 7 isolates (9.3%) belonged to the mammary pathogenic group and 5 isolates (15.7%) belonged to the fecal strains group. Of these ESBL-producing *E. coli*, the prevalence of *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub> was 83.33% and 33.33%, respectively. Also, 3 isolates (25%) carry both *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> genes at the same time. None of the isolates with positive ESBL phenotype carried *bla*<sub>SHV</sub> and *bla*<sub>cmv</sub> genes.

### CONCLUSION

Fecal strains can be a potential source of *E. coli* carrying resistance genes that may spread to the environment or food chain, and could pose serious and catastrophic health risks. Authorities should cling to the concept of One Health to minimize the risk of new varieties.

**Keywords:** Antibiotic resistance, *Escherichia coli*, ESBL, Bovine mastitis,

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Nanofilms as antimicrobial protection in medical industry

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Biomaterials are recently considerable in antimicrobial activities or the treatment of infections. Nanofilms are a tape-like gathering of nanomaterials, which could have antimicrobial, anti-biofilm, anti-inflammatory and pro-angiogenesis activities by inhibiting the adherence of bacteria to surfaces including tissues and medical devices. These biomaterials could also have drug delivery performances as like as antibiotic delivery to infected organs. In this study, we aim to review the activities of nanofilms in preventing or the treatment of infections in last decade.

#### MATERIALS AND METHODS

Google scholar and Pubmed were explored by the collection of 10 keywords according to Medical Subject Headings (MeSH). The search results were limited to the last 10 years (from 2013 to 2023) and lastly, all the relative articles were collected and reviewed.

#### RESULTS AND DISCUSSION

Investigated medical nanofilms were mainly made of chitosan, chlorhexidine, titanium oxide, poly-L-lactic acid, cooper, silver, carboxymethyl cellulose and iron-silver-tannic acid. Their applications are described as a protection in preventing of some infectious diseases such as wound infections, and also antibiotic drug deliveries. They could be used in sealing of invasive medical devices such as catheters, surgical devices and surgical sutures. Also, covering of hospital setting by antimicrobial nanofilms could prevent the hospital-acquired infections in hospitalized patients.

#### CONCLUSION

Nanomaterials including nanofilms are suitable agents in preventing or treating the infections with stable materials. They are also useful for drug or antibiotic deliveries in infected patients. It is recommended the investigations on the impact of nanofilms on the microbial involvement in other medical and surgical devices such as artificial heart valves and endotracheal, endoscopy or colonoscopy tubes, to be accurately carried out.

**Keywords:** Nanofilms, Nanomaterials, Nanotechnology, Infections, Anti-infective agents

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation of nitrite oxidoreductase (NOR) expression in an enriched NOB microbiome at different temperatures

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nitrite oxidizing bacteria (NOB) will oxidize nitrite to nitrate (NO<sup>3-</sup>), using the enzyme called nitrite oxidoreductase (NXR). The aim of this study was investigation total NOR expression in a microbiome containing enriched NOB bacteria.

### MATERIALS AND METHODS

Enriched microbial communities containing 6.1% *Nitrobacter winogradskyi* were prepared and transferred to NOB liquid media. Nitrite reduction and NXR gene expression were then measured at different temperatures. Specific q-PCR primers for NXR genes were designed for NOB strains, respectively. In addition, a constitutively expressed gene (16S rRNA gene) was used as an internal reference for selected strains. RNA was extracted from samples of microbial communities adapted to different temperatures and stored at -80 °C. cDNA was synthesized from the isolated RNA. Expression of NOR genes was analyzed by q-PCR.

### RESULTS AND DISCUSSION

To the expression of key NXR genes involved in these respective processes. The highest expression of NXR occurred in a temperature range of 25-30 °C. Nitrite is reduced to nitrate by the enzyme NXR, whose differential expression was detected in the microbiome. NXR expression correlated very well with community nitrite degradation activity

### CONCLUSION

In this study, we report an enriched NOB bacterial microbial community that removes nitrite at different temperatures so that it is proposed for water with high nitrite concentration for bioremediation systems, especially for aquaculture wastewater.

**Keywords:** Nitrite oxidizing bacteria (NOB), nitrite oxidoreductase (NXR), microbial community, aquaculture

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antimicrobial Effect of *B. subtilis*, against *Aeromonas hydrophila* and *Pseudomonas fluorescens*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

This research was conducted with the aim of using gamma radiation to generate mutant strains of *Bacillus subtilis* ATCC 6633 to study probiotic performance under in vitro conditions.

### MATERIALS AND METHODS

Strain *B. subtilis* ATCC 6633 was irradiated with gamma irradiation (6.25 KGy). Subsequently, the mutant strains were evaluated for their bacterial growth performance and antibacterial activity against some aquaculture pathogenic strains, *Aeromonas hydrophila* and *Pseudomonas fluorescens*.

### RESULTS AND DISCUSSION

The results of this study showed that strains number 45, 51 and 88 had a higher growth rate than the other sub-strains. The strongest antagonistic activity against the pathogens *A. hydrophila* and *P. fluorescens* was associated with number 45, while numbers 32 and 44 showed the least antagonistic activity compared to the others.

### CONCLUSION

In the present study, the generation of the mutant strain ATCC 6633 by gamma irradiation increased the antagonistic activity against the important pathogenic bacteria in fish culture against *A. hydrophila* and *P. fluorescens*. According to the results, substrain No. 45 should be evaluated for further studies in fish aquaculture systems.

**Keywords:** *B. subtilis*, Mutant strains, gamma irradiation, antimicrobial activity, aquaculture

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The mutant sub strain of *Bacillus subtilis* has the ability to remove ammonium in a RAS trout system

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### ABSTRACT

### BACKGROUND AND OBJECTIVES

The purpose of this research was to apply mutant substrains of *Bacillus subtilis* with high ability to remove ammonium and use in trout culture water under cold and laboratory conditions.

### MATERIALS AND METHODS

The effective dose of 5.8 kGy of the *Bacillus subtilis* strain was achieved by gamma radiation. Then 200 colonies were randomly selected and numbered. The potential for ammonium removal was investigated separately in the selected colonies. After the screening steps, the selected strains were adapted to cold temperatures. The biofilter water of a RAS trout system was prepared and the selected mutant substrain number 180 with a dilution of 10<sup>8</sup> CFU/ml was added to the trout RAS biofilter water and the ammonium concentration was evaluated.

### RESULTS AND DISCUSSION

In this study, *Bacillus subtilis* mutants were subjected. During fish farming, the system RAS moves towards nitrifying bacteria that have adapted to the system. Biofilters play an essential role in these systems and the most common microbial communities in these systems include *Nitrosomonas* sp., *Nitrospira* spp. *Nitrobacter* sp. and *Bacillus*. In this study, we report a strategy for biofortification of RAS biofilters enriched with a mutant substrain of heterotrophic bacteria. With this substrain, we achieved an improvement in ammonium removal compared to conventional communities in biofilters at 9 and 15 °C.

### CONCLUSION

*Bacillus subtilis* substrain number 180 has been proposed as an effective ammonium-degrading microorganism in trout fish RAS.

**Keywords:** *Bacillus Subtilis*, Mutation, Ammonium Oxidizing Bacteria, Cold Adaptation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Canine toxoplasmosis, updates on venereal transmission

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Toxoplasma gondii* is an important zoonotic intracellular protozoan parasite, which can affect all warm-blooded mammals and birds throughout the world, including humans. The cat is the definitive host but other warm-blooded animals such as humans and dogs are considered intermediate hosts.

Dogs can be a transport host for *T. gondii* oocysts, and can become infected by the ingestion of *T. gondii* oocysts from cat feces or by the feeding habit of uncooked mutton, however there are little information about venereal transmission of disease. Little information has been presented about placental transmission of toxoplasmosis in dogs, although it appears to be less common than in species such as cats, humans, and sheep, but congenital toxoplasmosis also occurs naturally in dogs.

In different studies *T. gondii* detected in the semen and reproductive organs of experimentally infected male rat, rabbit, goat, sheep, cattle, pig and dogs and there is some evidence propose that *T. gondii* can transmit with semen to female animals.

In this study, at the first step serological surveillance was done by MAT method in dogs which is selected from kennels with reproductive disorders compliant from the southeast of Iran and the overall infection rate was %43.5. At the second step, by using the most sensitive detection method "RE-based nested PCR assay" the probability of the presence of *T. gondii* were detected in the semen of 48 male seropositive dogs which 3 positive dogs (6.25%) was detected. The results of this project confirmed the venereal risk of toxoplasmosis transmission in dogs.

For the first time, tachyzoites were isolated from seminal fluid of experimentally infected male dogs and it has clearly showed that *T. gondii* is transmitted through semen to female dog. Vertical transmission of *T. gondii*, fetal reabsorption and congenial toxoplasmosis with cerebral cysts in puppies were also reported.

Venereal transmission can cause abortion and still birth in female dog and severe health problems for survived infected puppies. Hypothalamic-pituitary axis dysfunction was reported in murine toxoplasmosis in male rats, so further investigation must be done in infected male dogs to evaluate the effect of toxoplasmosis on the spermatogenesis and hormonal impairment.

**Keywords: Dog, PCR, Semen, Toxoplasmosis, Venereal transmission**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Photodynamic inactivation of antibiotic resistant microorganisms based on natural pigment Hypericin

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

One of the most important public health threats of the present century is antibiotic resistance. Due to the inability of antibiotics for mutagenic strains, there is an incremental increase in hospital admission and death rates. In this regard, the development of novel therapeutic modalities may be effective in controlling antibiotic resistance increase. Antimicrobial photodynamic therapy (aPDT) has been suggested as an alternative to elude the use of drugs that cause resistance. The aPDT is a non-invasive method utilizing a combination of a nontoxic dye known as a photosensitizer, visible light, and molecular oxygen. The ROS produced after the excitation of photosensitizer by light at the appropriate wavelength react with cellular macromolecules leading to destroy pathogenic microorganisms. Hypericin (perihydroxylated perylene quinone-based) is a natural pigment extracted from plants of the genus *Hypericum*. This photosensitizer has low dark toxicity and a high quantum yield of singlet oxygen production which makes it a promising candidate for photodynamic therapy. This review aimed to give the latest results about the Hypericin-mediated aPDT as a novel antimicrobial approach.

#### MATERIALS AND METHODS

This study was a systematic search up to July 2023, using large citation databases, including Scopus, the Cochrane Library, Web of Science, and Pubmed, to review the investigations performed on photodynamic therapy with Hypericin in different in vitro and in vivo conditions. In addition, the potential of this procedure for the treatment of antibiotic-resistant microbial infections was evaluated.

#### RESULTS AND DISCUSSION

The different studies have indicated that Hypericin has a strong potential to photoinactivate many microbes in planktonic and biofilm forms. Hypericin-based PDT exerts its antimicrobial effect both by direct destruction of microorganisms and the activation of the host's innate immune system. The increase of Hypericin concentration as well as a high light dose used for Hypericin photoactivation improves the efficacy of aPDT. The findings showed that Gram-positive strains such as *Staphylococcus* sp. and *Enterococcus* spp. are more susceptible to aPDT than Gram-negative bacteria including *Klebsiella pneumoniae* and *E. coli*. Several articles reported that Hypericin shows significant cytotoxic activity against methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) *S. aureus* isolates. In the main, the induction of cell death after aPDT is due to the overproduction of ROS inside the cells, resulting in severe plasma membrane damage and photooxidation of important biomacromolecules. The results of different studies indicated that Hypericin had an inhibition effect on the transcriptional regulator SarA expression and increased  $\beta$ -lactam efficiency in MRSA. Recent reports also showed that this photosensitizer can significantly decrease the minimum inhibitor concentrations of used antibiotics, especially at lower light doses. Compared to other natural photosensitizers, Hypericin presents a more effective antimicrobial effect, because some studies showed that it inhibited *E. coli* growth to 99.9% after photoactivation with a light dose of 6 J/cm<sup>2</sup>, while curcumin achieved suppression of the growth of bacteria with 90% efficacy after using light doses higher than 10 J/cm<sup>2</sup>.

#### CONCLUSION

Based on the current literature, it can be concluded that Hypericin-mediated aPDT can be considered a promising approach to cure antibiotic-resistant microbial diseases.

**Keywords:** Hypericin, Antimicrobial approach, Photodynamic therapy, Antibiotic resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Detection of *Rickettsia* genus in collected ticks from hedgehogs of Kerman

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

Nowadays many emerging zoonotic microbial diseases are threatening public health. Rickettsiosis, which is caused by different species of rickettsiae is mostly transmitted by *Rhipicephalus sanguines* and *Rhipicephalus turanicus* and *Rickettsia massillie* and *Rickettsia sibirica* are among the most prevalent and pathogenic rickettsia all around the world. Rickettsiosis in humans and animals may cause general symptoms such as subcutaneous bleeding, muscle pain, fever and other symptoms. Considering the numerous reports of human infections and also the frequency of domestic and wild animals with tick infestation, it is necessary to investigate the prevalence of the disease in provinces such as Kerman with confirmed cases of human infection. Recently, wild urbanized animals such as hedgehogs have come close to urban areas due to their territory destruction and ecosystem changes, and have been considered as potent reservoirs for many zoonotic diseases.

#### MATERIALS AND METHODS

In this study, during six month period, long-eared and brandt's hedgehogs were live-trapped, 45 animals with severe tick infestation were selected as the study population, and their external parasites were isolated. After extracting their DNA, the samples were evaluated using *Rickettsia* specific primers and real time PCR technique.

#### RESULTS AND DISCUSSION

Finally, out of a total of 45 collected samples of isolated ticks (at least 5 ticks from each hedgehog) 5 pool samples (11.11%) were positive for *Rickettsia* genus including 2 samples containing *Rickettsia sibirica*, 2 samples containing *Rickettsia rhipicephali* and one sample with *Rickettsia massilliae* and *Rickettsia rhipicephali* co-infection.

#### CONCLUSION

Considering the notable positive results of infected ticks collected from hedgehogs as free roaming animals it can be concluded that the investigation of animal reservoir's for rickettsial agents in different parts of Iran is of great importance. The present study, can provide the basis for further investigation in this field as detection of main animal reservoirs are critical for suggestions of efficient control and prevention strategies.

**Keywords:** *Rickettsia rhipicephali*, *Rickettsia sibirica*, *Rickettsia massilliae*, *Rhipicephalus sanguineus*, *Rhipicephalus turanicus*, Hedgehog.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Characteristics of the bacterial isolates collected from hospitalized patients with ventilator-associated pneumonia

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Ventilator-associated pneumonia (VAP) is a type of nosocomial infection which involve hospitalized patients in Intensive Care Units (ICUs) using mechanical ventilation for at least 72 hours. Endotracheal tube (ETT) is a place for the colonization of microbial populations and subsequent biofilm formation leading to relative drug resistance. Also, it is a direct route for the transmission of the causing bacteria to the lungs. In this study, we aim to identify the microbial cause of VAP and evaluating the antibiotic resistance patterns.

### MATERIALS AND METHODS

Sampling was done using ETT suction among hospitalized patients in selected hospitals in Urmia, Iran. Then, specimens were cultured in microbiological media and further differential and biochemical tests were performed. After the identification of the isolates, Antimicrobial Susceptibility Testing (AST) was conducted using disk diffusion for selected antibiotics according to Clinical and Laboratory Standards Institute 2022 (CLSI 2022) guideline. Finally, non-fermentative gram-negative bacilli were tested for the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for colistin.

### RESULTS AND DISCUSSION

Most of VAP patients were male with the mean age of 65 years old. Clinical findings of the patients were mostly included of fever and dyspnea with at least one period of antibiotic therapy. Also, drug abusers had assigned the most predisposing conditions. Out of 43 collected specimens, 40 of them were VAP positive, which identified as various gram-negative bacteria. Most of the isolates (45%), belonged to *Acinetobacter spp.* AST results showed that most of isolates were resistant to ciprofloxacin and sensitive to amikacin. About 72% and 22% of *Acinetobacter spp.*, were extensively drug-resistant (XDR) and multidrug-resistant (MDR), respectively. MIC results were shown that about 78% of *Pseudomonas aeruginosa* and *Acinetobacter spp.* were intermediate and about 22% were resistant to colistin. Finally, the MBC results showed that 64 µl/ml and 0.5 µl/ml were the highest and the lowest concentration in bactericidal activities and 81% of tested isolates had the MIC value equal to the MBC value.

### CONCLUSION

VAP is a critical infection and according to the findings, there is high drug resistance among bacterial cause of VAP, which needs necessary new insights for preventing and controlling this healthcare problem.

**Keywords:** Ventilator-associated pneumonia, Drug resistance, *Acinetobacter*, Colistin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Biomarkers as rapid screening tests for the detection of sepsis

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The infection in blood stream, known as sepsis, is one of the most critical infections which needs immediate detection and subsequent medical treatments. The routine microbiological tests are time consuming. So, using a set of biomolecules named as “panel of biomarkers” is an alternative and rapid way in order to diagnosis of infection. In this review, we aimed to describe some important biomarkers for the screening of sepsis.

#### MATERIALS AND METHODS

Available international databases, Google scholar, Pubmed and Springer link were explored. The given keywords by Medical Subject Headings (MeSH) such as “Biomarkers” and “Sepsis” were also used in searching papers. Finally, the results have been limited to last 10 years, from 2013 to 2023, and related articles were analyzed.

#### RESULTS AND DISCUSSION

The findings exhibit that the biomarkers of sepsis are mainly classified in two categories of general and specific biomarkers. General biomarkers are Erythrocyte Sedimentation Rate (ESR), white blood cells count and Ferritin which all have low-sensitivity in diagnosis of sepsis. The higher sensitivity and specificity are associated to specific biomarkers which categorized in five groups: Acute-phase proteins (Procalcitonin (PCT), C-reactive protein (CRP), lipopolysaccharide-binding protein (LBP), etc.), cytokines (Il-1, Il-6, Il-8 and TNF- $\alpha$ ), coagulation biomarkers (antithrombin, proteins C and S, D-dimers, etc.), complement proteins (C3b and C5a) and lastly, soluble receptors (sTREM-1 and suPAR). Among all of these biomarkers, procalcitonin was the most important and useful biomarker, and it has its effectiveness in cooperation with some other selective biomolecules such as CRP and sTREM-1.

#### CONCLUSION

None of the mentioned biomarkers is definitive and applicable alone. Thus, a panel of biomolecules is necessary for early diagnosis of sepsis and subsequently, starting the first line wide-spectrum antibiotics to prevent the expansion of infection until the exact identification and antibiogram testing results are obtained.

**Keywords:** Sepsis, Biomarkers, Acute-phase proteins, Cytokines, Blood coagulation factors, Complement system proteins.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Anti-tumor effects of *Bacteroides fragilis* and *Bifidobacterium bifidum* culture supernatants on mouse breast cancer

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Breast cancer is one of the most important causes of cancer related morbidity and mortality in the world. Along with genetic, environmental factors also play a multifaceted role in the development of disease. *Bacteroides* and *Bifidobacterium*, as microbial flour, play pivotal role against breast cancer development in vivo and in vitro.

#### MATERIALS AND METHODS

In this study we evaluated the anti-proliferative and anti-tumor effects of *Bacteroides fragilis* (F.S), *Bifidobacterium bifidum* (B.S) and *Bifidobacterium bifidum* + *Bacteroides fragilis* supernatants (B.F.S) in in vivo and in vitro model of 4T1 cell line breast cancer. Different doses of bacterial supernatants assessed in both model. The effects of culture supernatants were determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Tumor size/volume, cell growth inhibition and cytokine secretion were assessed.

#### RESULTS AND DISCUSSION

Our results indicated that the relative tumor volume significantly increased in the mice receiving intratumoral (I.T) PBS ( $p < 0.05$ ). Surprisingly, 3 out of the 4 mice were cleared from the tumor thoroughly in 10–25 days after intratumoral administration of B.F.S. Quantification of cytokines clearly showed that the mice receiving intratumoral B.F.S significantly secreted higher interferon  $\gamma$  (IFN- $\gamma$ ) and level and the other side, secreted lowest IL-10 level compared with the other groups ( $p < 0.05$ ). Overall, our findings indicated that intratumoral administration of B.F.S effectively inhibited the growth of breast tumors through induction of necrosis and suppressing proliferation and angiogenesis.

#### CONCLUSION

Collectively, our study showed that bacterial supernatants (F.S, B.S, F.B.S) can act as potent Anticancer agents, they can inhibit the growth of breast cancer cells in vitro, on the other side, they reduce the tumor volume and the recovery process increases in mice bearing breast tumor.

**Keywords:** Breast cancer, Flour microbiota, *Bacteroides fragilis*, *Bifidobacterium bifidum*, culture supernatant

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigation of bacterial infection agents of burn wounds, determining their antibiotic resistance pattern and producing metallo-beta-lactamase.

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

Most of the deaths that occur due to burns are due to contamination of the wound site and infection. Up to date information on the bacterial agents of infection and their antimicrobial patterns play an important role in the control and treatment of burn infections. Determination of bacteria that infect burn wounds, the antibiotic resistance pattern of each bacteria and production of metallo-beta-lactamase enzymes in these isolates is the target in this project.

##### MATERIALS AND METHODS

In this study, 78 samples of burn wound secretion of patients admitted to the burn hospital in Ahvaz city were examined in a period of 6 months. The isolates were identified using conventional biochemical tests. The drug sensitivity of the isolates was determined using the disk diffusion method and based on the Clinical Laboratory Standards Institute (CLSI) table resistance pattern of each isolate was determined.

##### RESULTS AND DISCUSSION

The total number of samples was 78, of which 119 isolates were isolated. 71.79% of the samples were men and 28.20% of the samples were women. *Pseudomonas aeruginosa* was the most common microorganism isolated with a frequency of 37.81%. *Proteus sp.* with a frequency of 22.68%, *Staphylococcus sp.* with a frequency of 7.56%, *Acinetobacter sp.* with a frequency of 4.2% and *Klebsiella sp.* with a frequency of 2.52% were in the next ranks. It should be noted that the results of antibiogram and polymerase chain reaction (PCR) are being analyzed and reviewed.

##### CONCLUSION

This study shows the change in many bacterial agents isolated from burn wound as well as the high level of antibiotic resistance of isolates. Therefore methods to control the spread of strains with multiple antibiotic resistance and new methods to treat burn contamination should be considered.

**Keywords: Bacterial Infections, Burn Department, Antibiotic Resistance**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and characterization of *Lacticaseibacillus casei* RIGLD MG-1 from the gastric microbiome of a healthy human subject

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVE

Probiotics are beneficial organisms that have been extensively studied for their positive effects on human health. *Lactobacillus* bacteria are one of the most frequently consumed probiotic species and can colonize various niches in the human gastrointestinal tract. This study aimed to isolate and characterize *Lactobacillus* strains from the gastric microbiome of healthy human subjects.

#### MATERIALS AND METHODS

Stomach biopsies were obtained from healthy individuals undergoing routine endoscopic procedures at Taleghani Hospital in Tehran. The collected samples were incubated in selective De Man, Rogosa and Sharpe agar (MRS) under anaerobic conditions. Isolated colonies were then identified using colony morphology, and Gram staining, followed by PCR and 16S rRNA gene sequencing. The isolated *Lactobacillus* strains were further characterized for their safety, functional properties, and antibiotic susceptibility testing using disk diffusion method.

#### RESULTS AND DISCUSSION

We successfully isolated and identified a strain of *L. casei* RIGLD MG-1 from the gastric biopsies of a healthy human. The strain exhibited no hemolytic, gelatinase, and DNase activities and high tolerance towards acid and bile salts. Furthermore, this strain was sensitive to ampicillin, erythromycin, clindamycin, tetracycline, and chloramphenicol and resistant to vancomycin, kanamycin, streptomycin, and nalidixic acid.

#### CONCLUSION

In conclusion, our results demonstrated that the isolated *L. casei* RIGLD MG-1 was safe and tolerant to harsh gastric environment, and potentially could be considered as a probiotic strain. Further research is required to assess the antimicrobial and immunomodulatory activity and beneficial effects of this strain on stomach-related diseases and *Helicobacter pylori* infection.

**Keywords:** Gastric microbiome, Probiotic, *Lactobacillus*, Isolation, Safety assessment

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The mechanisms of drug resistance in Multidrug-resistant *Neisseria gonorrhoeae*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Gonorrhea is the second prevalent sexually transmitted infection (STI) around the world which caused by *Neisseria gonorrhoeae* (*N. gonorrhoeae*). Recent reports exhibit the high-resistance of this bacterium against the main antibiotics: Penicillin, cefixime, ceftriaxone, tetracycline, azithromycin and ciprofloxacin with the globally increase in the prevalence of 3.8% within last decade. In this review, we will discuss the main mechanisms of drug resistance in multidrug-resistant *Neisseria gonorrhoeae* (MDR-NG) that are those strains with resistance to the mentioned antibiotics.

### MATERIALS AND METHODS

Google scholar, Pubmed, Scopus and Springer link were explored according to the given keywords by Medical Subject Headings (MeSH): “*Neisseria gonorrhoeae*”, “drug resistance”, “penicillin”, “cefixime”, “ceftriaxone”, “tetracycline”, “azithromycin” and “ciprofloxacin”. The results were limited to the last decade from 2013 to 2023., and lastly, related original papers were collected.

### RESULTS AND DISCUSSION

Findings illustrate that chromosomal expression of *mtrCDE*, synthesis of penicillin-binding proteins (PBPs), substitutions in PenA, PorB1b and PonA in penicillin and oxyimino-cephalosporin Resistance, plasmid-mediated expression of *tetM* and chromosomal Single Nucleotide Polymorphisms (SNPs) in *mtrR*, *porB* and *rpsJ* genes in tetracycline resistance, SNPs in 23S rRNA (C2611T and A2059G), *mtrR* mutations, expression of *ermBCF* genes and existence of MacAB and *mef*-encoded efflux pumps in azithromycin resistance and lastly, *gyrA* SNPs (S91F, D95N, D95G etc.) and *ParC* SNPs (D86N, S88P, and E91K) in ciprofloxacin resistance, are the main cause of increased Minimum Inhibitory Concentrations (MICs) and the drug resistance in MDR-NG.

### CONCLUSION

Due to the expansion of the resistance of *N. gonorrhoeae*, as one of the most prevalent pathogens to the most effective treatments, it is necessary to discovery some novel antibacterial agents or vaccine candidates in order to control the future possible crisis.

**Keywords:** *Neisseria gonorrhoeae*, Multidrug resistance, Beta-Lactams, Tetracycline, Macrolides, Fluoroquinolones

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Characterization of antimicrobial activities of *Bifidobacterium lactis* BB12 and their inhibitory effect against some foodborne pathogens

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Probiotics, such as *Bifidobacterium animalis* BB-12, have gained much interest because of their possible health advantages, including their function in reducing the spread of infections that can be found in food. In this study, we examined the effect of the BB-12 strain and its supernatant on *Listeria monocytogenes* and *Salmonella enterica* subsp. *enterica* serotype Typhimurium growth.

#### MATERIALS AND METHODS

Agar well diffusion, disk diffusion, minimum inhibitory concentration (MIC) tests and BCA protein test were used to evaluate the probiotic's antimicrobial activity and supernatants protein concentration.

#### RESULTS AND DISCUSSION

The results showed that the tested pathogens were significantly inhibited by the probiotic ( $p < 0.05$ ). Additionally, after 24 and 48 hours of incubation, the BB-12 strain supernatant demonstrated significant antibacterial effect against both pathogens ( $p < 0.05$ ). A BCA protein test on the cell-free supernatant was carried out to investigate the underlying processes, and the results showed that the 24-hour culture supernatant contained a significant quantity of protein. This implies that the protein components in the supernatant could help explain its increased ability to combat infections.

#### CONCLUSION

The BB-12 strain and its released proteins as a viable strategy to combat foodborne pathogens, hence enhancing food safety and public health, is highlighted by these findings

**Keywords:** Foodborne Pathogens, Probiotics, Cell-Free Supernatant, *Bifidobacterium Animalis* BB-12

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and Evaluation of *Amycolatopsis* sp. strain 1119 as PGP strain in the Commercial Greenhouse of Cucumber, and the study of quality attributes of fruits

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The possibility of chemical fertilizer residues in cucumber has raised concerns about the consumption of this beneficial fruit. Although very good studies have been reported on the effect of growth-promoting bacteria on increasing the health of cucumber plants, their performance in commercial greenhouse conditions has been less studied. The aim of current study was the evaluation of the effect of *Amycolatopsis* sp. strain 1119 on cucumber fruit yield and quality under commercial greenhouse conditions.

#### MATERIALS AND METHODS

In this study, the *Amycolatopsis* sp. strain 1119 (Accession No: MG984616) was isolated from a maize field. PGP traits like Presumptive phosphate-solubilizing activity, auxin, and siderophore production, and antagonistic activity against pathogenic organisms were investigated. Greenhouse experiments were conducted in a 1250 square meter commercial greenhouse located in Varamin County, Tehran Province, Iran. The experimental design was a randomized complete block (RCB) with two treatments and four replicates. The experiment groups were treated with 5 g per plant sand containing *Amycolatopsis* sp. strain 1119. One hundred days after treatment, 15 times (with a two-day interval), fruits were harvested, and their fresh weight, nitrate content, dry weight, and total soluble sugar content were evaluated. For sensorial evaluation, fruits were presented to 20 untrained volunteers (ten males and ten females in the range of 24–50 years old). Each participant was asked to evaluate fruits by scoring characteristics (bitterness, fragility, aroma, juiciness, appearance, flavor, and overall acceptance).

#### RESULTS AND DISCUSSION

*Amycolatopsis* sp. strain 1119 was isolated from a maize field and possessed great antagonistic activity against *Phytophthora capsici*, *Phytophthora drechsleri*, *Pythium ultimum*, *Rhizoctonia*

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*solani*, and *Fusarium oxysporum*. PGP activity of this strain is related to the production of Indole-3-acetic acid (IAA) and siderophore, positive for the ability to grow on a nitrogen free medium, and its good hydrolytic enzyme activity like cellulase and chitinase. Also, the strain can solubilize tricalcium phosphate.

However, the fruit yield was 3.9 tons/1000 m<sup>2</sup> in the control group, the yield of experiment group under treatment of 1119 isolate increased 20% (4.7 tons/1000 m<sup>2</sup>) in one month. The results showed that soil treatment with 1119 strain significantly reduced fruit dry weight percentage (31%) and total soluble sugar content (14%) compared to control. Decreased fruit nitrate content was another effect of this strain on fruit quality. The content of cucumber nitrate in the bacterial treatment was 9.58 mg/100 g fresh fruit weight, less than one-third of the control group. Sensory evaluation showed that the increase in the aroma, flavor, and fruit juice of the cucumber fruits in plants treated by 1119 isolate has a higher overall acceptance score compared to the control.

### CONCLUSION

Based on the results of many researches, manipulation of the cropping system and artificial introduction of PGPs into the soil as biofertilizer is a practical method to reduce the consumption of harmful chemical fertilizers. Here, for the first time, we showed the different effects of *Amycolatopsis* on cucumber. Our results demonstrated that the *Amycolatopsis* strain 1119 has great potential to be used as an active principle for bio-inoculant development because of cucumber fruit yield improvement in a commercial greenhouse. On the other hand, nitrate in the human digestive system reduces to nitrite, which is a harmful product for humans and other organisms, and the restriction of its consumption is necessary. The use of PGPs by the ability to reduce the nitrate content can be a great potential fertilizer to replace chemical fertilizers with various environmental side effects.

**Keywords:** PGPB, *Amycolatopsis*, Cucumber fruit, Fruit quality improvement

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# An overview of nosocomial bacterial infections: epidemiology, control, and antibiotic resistance

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nosocomial infections or healthcare-associated infections occur in patients under medical care. Frequently prevalent infections include central line-associated bloodstream infections, catheter-associated urinary tract infections, surgical site infections and ventilator-associated pneumonia. Nosocomial infections can be managed by practicing infection control programs. This study aims to provide a detailed review of literary studies to discover the outbreak of nosocomial infections and antibiotic resistance especially in Middle Eastern countries.

### MATERIALS AND METHODS

Studies related to diverse facets of nosocomial infections and bacterial resistance that were published from 2000 to 2023 were sought by conducting comprehensive searches in databases like PubMed, Medline, Medscape, Cochran Library, WHO, CDC, Scopus, Osmosis of Elsevier, and Google Scholar. In order to obtain information on nosocomial infections and pathogens, a keyword search was carried out encompassing terms of Nosocomial Infections, Antibiotics, Antibiotic Resistance, Healthcare, Pathogens, and Infection-Control Strategies. Upon completion of this search process, out of the total of 205 retrieved studies through these keywords, 40 were considered relevant for further review.

### RESULTS AND DISCUSSION

Nosocomial infections and antibiotic resistance were considered to become emerging problems in Middle Eastern countries, causing serious mortality and morbidity. In this regard, *Escherichia coli* and *Klebsiella* species among gram-negative bacteria, and *Staphylococcus aureus* among gram-positive bacteria were stated as the most observed pathogens activated in nosocomial infections. In addition, multi-drug resistant (MDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are critical priority pathogens that cause nosocomial infections. High antibiotic resistance among hospital pathogens is reported to be one of the most important challenges so they are commonly resistant to the most important classes of antibiotics including penicillin, cephalosporins, carbapenems, and fluoroquinolones antibiotics.

### CONCLUSION

In order to control nosocomial infections, government officials are recommended to ensure that hospitals follow standard nosocomial infection control strategies and guidelines.

**Keywords:** Infection-Control Strategies, Nosocomial Infections, Antibiotic Resistance, Pathogens, Microbiology

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and identification of *Cutibacterium acnes* from skin lesions

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The aim of this investigation was to isolate *Cutibacterium acnes* from comedones, deep facial cysts, and identify them by diagnostic-differential tests.

#### MATERIALS AND METHODS

Samples were collected from different areas of the skin surface of 50 patients aged 18 to 60 years old having symptoms of acne vulgaris. Ethics consents were obtained (Ethics ID: IR.UI.REC.1399.057). Samples were transferred to sterile tubes containing thioglycolate and tryptic soy broths containing 0.5 µg/ml of vitamin K<sub>1</sub> and were covered with 1 ml of sterile mineral oil. The obtained samples were streak cultured on Brucella agar and blood agar medium. The plates were then placed in an anaerobic jar chamber in the presence of a Gas-pack type A and incubated at 35-37 °C for 3-6 days. Genomic DNA was extracted. Specific primers were used to amplify *recA* and *MmdA* genes in *Cutibacterium* species.

#### RESULTS AND DISCUSSION

White colonies with a viscous and elastic appearance were observed, and based on Gram staining; the bacteria were in the form of gram-positive polymorphic rods, pairs or short chains. The length of the PCR fragment to identify the *recA* and *MmdA* genes was equal to 334 and 633 base pairs, respectively. Then, the obtained sequences were analyzed using BLAST tool in NCBI database and registered in GenBank (accession numbers: LC542930.1, LC542932.1, LC571584). In this regard, the phylogeny relationship was investigated based on the *recA* gene nucleotide sequence.

#### CONCLUSION

Based on the obtained results, *C. acnes* (accession numbers: LC542930.1 and LC542932.1) were closely related to *Propionibacterium granulosum*, reported from France with Accession no. (AY883046.1). Although both belong to the *Cutibacterium* genus, they belong to two different species. As a result, this issue shows various strains distributing in Iran, which can be investigated more precisely by examining more samples.

**Keywords:** Acne, *Cutibacterium acnes*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Introducing the active substance cinnamaldehyde in *Cinnamomum verum* plant and investigating its antimicrobial properties against pathogenic bacteria

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The spread of antibiotic resistance, especially in gram-negative bacteria, has led to the use of the combined effect of antibiotics and obtaining herbal antibiotic compounds in the treatment of infections caused by these bacteria. The purpose of this study is to investigate the effect of the active substance cinnamaldehyde present in the *Cinnamomum verum* plant on *Pseudomonas aeruginosa* under in-vitro conditions, to detect and report the antimicrobial and inhibitory effect on *P. aeruginosa* bacteria.

#### MATERIALS AND METHODS

The active substance cinnamaldehyde was purchased from Sigma-Aldrich. *P. aeruginosa* isolates were separated from the clinical samples of patients referred to the laboratory of Razi Hospital in Rasht using different biochemical methods as well as Gram staining method. In this research, 30 clinical strains of *P. aeruginosa* along with the standard strain ATCC27853 were investigated. Antibiogram test (Kirby-Bauer) and quantitative minimum concentration test were used to check the antibacterial effects by successive dilutions of MIC and MBC, and finally, the diameters of the lack of growth around the discs impregnated with 1 mg/ml of the effective herbal substance cinnamaldehyde were measured and recorded with a ruler.

#### RESULTS AND DISCUSSION

In this research, the minimum diameter of the non-growth halo of the isolates is reported to be 13 and the maximum diameter is 22 mm (average 16.83 mm) and the average of MIC and MBC cinnamaldehyde on the isolates was 0.26 and 0.52 mg/ml, respectively.

#### CONCLUSION

The results of this study showed that cinnamaldehyde can be used as a suitable candidate with good antimicrobial effects in antibacterial drugs and treatment of infections.

**Keywords:** *P. aeruginosa*, cinnamaldehyde, *Cinnamomum*, Antimicrobial Effects, Infection.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Insight to *Acinetobacter baumannii*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Acinetobacter baumannii* is the most important member associated with hospital-acquired infections worldwide. This aerobic Gram-negative coccobacillus had been regarded as a low-grade pathogen, but it is a successful pathogen responsible for opportunistic infections of the skin, bloodstream, urinary tract, and other soft tissues. Several virulence factors have been identified by genomic and phenotypic analyses, including outer membrane porins, phospholipases, proteases, lipopolysaccharides (LPS), capsular polysaccharides, protein secretion systems, and iron-chelating systems. *A. baumannii* has a number of resistance mechanisms, including  $\beta$ -lactamases, aminoglycoside-modifying enzymes, efflux pumps, permeability defects, and modifications of target sites.

#### MATERIALS AND METHODS

The accumulation of several resistance mechanisms in *A. baumannii* has gradually decreased the number of antibiotic classes available to treat *A. baumannii* infections in clinical practice. *A. baumannii* is intrinsically resistant to a number of commonly used antibiotics, such as aminopenicillins, first- and second-generation cephalosporins. For susceptible organisms, first-line therapy consists of a carbapenem, such as imipenem-cilastatin, meropenem, or doripenem. Carbapenem resistance rates for *A. baumannii* have been rising dramatically worldwide.

#### RESULTS AND DISCUSSION

Colistin-based combination therapies have been preferred over colistin monotherapy given colistin's suboptimal pharmacokinetics and pharmacodynamics. Tigecycline, developed for the treatment of multidrug-resistant (MDR) pathogens and having potent in vitro activity against *A. baumannii*.

#### CONCLUSION

Phage therapy has gained particular importance for the treatment of bacterial infections. Single Phage Therapy: Therapies based on a single virus type, also known as monophage therapies, have been extensively applied as *A. baumannii* treatments. Phage-Encoded Enzymes for the Treatment of *A. baumannii*: Endolysins are phage-produced hydrolases that lyse bacterial cell walls, allowing the further release of progeny phages at the end of the replication cycle. Depolymerases are phage-derived enzymes that facilitate the early stages of phage infection by degrading extracellular bacterial protein. Phage-antibiotic synergy (PAS) refers to the usage of antibiotics at sublethal doses in combination with phage administration, with the aim of increasing the release of phage-progeny from bacterial cells. Cocktail Therapy: Phage cocktails typically consist of multiple phages combined, with each of them having unique host specificity due to selective affinity towards a specific bacterial receptor, conferring a broad therapeutic lysis spectrum.

**Keywords:** *Acinetobacter baumannii*, phage therapy, Antibiotic resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Frequency of diagnostic genes of *nuc* and *femA* in *Staphylococcus aureus* Strains Isolated from Patients Referring to Some Treatment Centers of Qom City, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Staphylococcus aureus* is one of the major etiology of contagious infections in the hospital and community (nosocomial infections). Resistant strains of this bacterium have high prevalence and fatality. Therefore, for antibiotic treatment and infection control improvement, it is necessary to identify these strains accurately and quickly and the occurrence of false positive and negative results as far as possible be avoided. The objective of this study was to isolate *S. aureus* bacterium using two important diagnostic genes, *nuc* and *femA*, by Multiplex PCR technique from isolated samples in patients referred to some treatment centers of Qom city.

### MATERIALS AND METHODS

In this descriptive cross-sectional study, 250 clinical specimens were collected during the eight-month period from January 2022 to August 2022 and then isolation and initial identification of *S. aureus* isolates (using standard bacteriology methods) the isolated strains were confirmed by Multiplex PCR technique and amplification of *nuc* and *femA* genes as a molecular diagnostic markers of *S. aureus*.

### RESULTS AND DISCUSSION

In total, 250 clinical specimens were prepared and evaluated from urine (98 samples), surgical wounds (112 samples), and blood (40 specimens). By using standard bacteriology tests, 90 strains of *S. aureus* (36%) were identified that of these, 70 strains were genotyped with *femA* and *nuc* genomes by Multiplex PCR technique.

### CONCLUSION

The results of this study showed that Multiplex PCR technique can help to better detect *S. aureus* strains and also prevent false positive results. Multiplex PCR can be used as a convenient, fast and high-precision method to replace common methods.

**Keywords:** *Staphylococcus aureus*; *femA* gene, *nuc* gene, Multiplex PCR technique.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluation of the CHAPK -SH3b endolysin effect on different strains of *Staphylococcus* by plate lysis assay

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Excessive use and misuse of antibiotics have led to the emergence of bacterial strains with multiple resistance, most of which are resistant to more than one type of antibiotic. The emergence of these resistant bacterial species highlights the need to develop alternative treatments. Meanwhile, bacteriophages (phages) and peptidoglycan-degrading enzymes derived from them (endolysins) have shown promising results as an alternative to antibiotics. Endolysins have a fast and unique action, high specificity to kill pathogens, low probability of developing bacterial resistance, and a distinct proteinaceous nature.

#### MATERIALS AND METHODS

In this project, A two-domain chimera recombinant protein composed of the CHAPK domain of endolysin LysK and SH3b domain of lysostaphin was used. The effect of CHAPK -SH3b on different serotypes of *Staphylococcus aureus* (sensitive and resistant to methicillin ) and other strains of *Staphylococcus* such as *Staphylococcus chromogenes*, *Staphylococcus cohnii*, *Staphylococcus epidermidis* and... was assessed with plate lysis assay. In brief, 10 µL of diluted endolysin was spotted on a freshly prepared bacterial lawn on TSA plates. Spotted plates were air-dried in a laminar flow hood and incubated overnight at 37°C. Cleared spots were digitally photographed within 20 h of plating the cells.

#### RESULTS AND DISCUSSION

CHAPK -SH3b not only displayed effective lytic activity against all tested methicillin-sensitive *staphylococcal* strains but was also effective on methicillin-resistant *staphylococcus aureus*. A difference in lytic ability was observed with different staphylococcal strains, possibly reflecting differences in the cell wall composition between strains. However, other gram-positive bacteria from different genera, including *Bacillus cereus* and *Enterococcus faecalis*, were unaffected by CHAPK -SH3b, suggesting that CHAPK -SH3b is specific to the genus *Staphylococcus*.

#### CONCLUSION

We reported a unique chimeric endolysin with robust lytic activity and an extended-spectrum host range against multiple staphylococcus species in vitro. The SH3b cell-binding domain of lysostaphin possesses an unusual binding mechanism that allows a synergistic and structurally dynamic recognition of *S. aureus* peptidoglycan, as the pentaglycine cross-bridge and the peptide stem are recognized by two independent binding sites located on opposite sides of the SH3b domain. This fact suggests that the lysostaphin SH3b cell-binding domain facilitates the catalytic domain to find its target bonds within the peptidoglycan, regardless of the presence of modifications in the matured peptidoglycan.

**Keywords: Histidine dependent Amidohydrolase Peptidase (CHAP), Chimeric protein, plate lysis assay, Endolysin, *staphylococcus aureus*, staphylococcal strains**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Distribution analysis of tetracycline resistance genes in fecal *Escherichia coli* isolated from sheep

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

There is a growing demand for livestock products and by-products due to an increase in the global human population. In this regard, the inappropriate use of antibiotics to enhance the growth of animals and reduce infectious diseases has led to the development of commensal and/or pathogenic bacteria resistant to antibiotics. One of these commonly used antibiotics is tetracycline. *Escherichia coli* (*E. coli*) is one of the important commensal bacteria with the ability to cause diseases, in which antibiotic resistance has been increasing worldwide. To better understand the selection pressures associated with the use of tetracycline in animal feed, the presence of six tetracycline resistance genes in fecal *E. coli* isolated from sheep was investigated.

##### MATERIALS AND METHODS

In this study, 30 stool samples were collected from sheep in Shiraz industrial slaughterhouse. All the samples were carefully placed in sterile containers and transferred to the microbiology laboratory as soon as possible. Samples were cultured on MacConkey agar medium and biochemical tests were performed to identify *E. coli* isolates. DNA of all confirmed *E. coli* isolates was extracted by boiling method, and polymerase chain reaction (PCR) assay was employed to evaluate the presence of *tetA*, *tetB*, *tetC*, *tetD*, *tetK*, and *tetM* genes in these isolates.

##### RESULTS AND DISCUSSION

Tetracyclines are antibiotics that are widely used in veterinary medicine; as a result, many bacteria - including *E. coli* - have become resistant to this drug. In the present study, 24 fecal *E. coli* isolates were obtained from the sheep. Among them, 15, 5, 1, 0, 14, and 10 isolates had *tetA*, *tetB*, *tetC*, *tetD*, *tetK*, and *tetM* genes, respectively. Tetracycline resistance genes with the highest frequency were *tetA* (62.5%), *tetK* (58.3%), and *tetM* (41.7%), respectively. In contrast, the *tetD* gene was not found in any of the isolates.

##### CONCLUSION

Tetracyclines are valuable antibiotics in both human and veterinary medicine, but the emergence of bacterial resistance against them has reduced their therapeutic effect. The relatively high frequencies of tetracycline resistance genes in the fecal *E. coli* isolates of the studied sheep confirm this problem and the risk caused by the transmission of these resistant bacteria to humans.

**Keywords:** Antibiotic resistance; *Escherichia coli*; Tetracycline; PCR; Sheep

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# MicroRNAs in pancreatic cancer: Their biogenesis, roles and diagnosis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Pancreatic cancer is one of the deadliest types of cancers. Cancer is the result of aberrant gene expression that affects various cell signaling pathways. Therefore, it can be said that the discovery of microRNAs, which are small non-coding RNA molecules that play a role in regulating gene expression, has provided a new opportunity to study this disease.

### MATERIALS AND METHODS

During miRNA biogenesis, miRNAs are subject to intense transcriptional and post-transcriptional regulation, and the elucidation of these mechanisms has improved our understanding of miRNA deregulation in disease. MiRNA genes are often located at genomic regions associated with cancer and also several researchers reported that abnormal expression of miRNA genes has been associated with cancer. Several experiments and clinic analysis suggest that miRNAs may function as a novel class of oncogenes or tumor suppressor genes, so they have been identified to act as tumor suppressors or as oncogenes based on their modulating effect on the expression of their target genes. Pancreatic cancer impinges profoundly on mankind and, is one of the most aggressive and fatal malignancies. A real-time, quantitative PCR assay was used to profile the expression of over 200 miRNA precursors in clinical specimens of pancreatic cancer and pancreatic cancer cell lines.

### RESULTS AND DISCUSSION

Pancreatic cancer is one of the worst-looking solid tumors, due to usually the late diagnosis and lack of effective therapy. Identification of markers characteristic of individual cancer phenotypes is strategic for early diagnosis and the use of effective therapeutic methods. Given that miRNAs are markers of the metabolic state, they can be used as a potent tool to monitor cancer progression and the effectiveness of cancer therapeutic.

### CONCLUSION

Understanding the roles of miRNAs can have a great beneficial impact on the early detection, diagnosis, treatment and prognosis of pancreatic cancer. Continuing research about their roles may bring new possibilities for anticancer therapy of this fatal disease.

**Keywords: MicroRNA, Gene regulation, Pancreatic cancer, Biogenesis, Diagnosis, Biomarkers**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Drug-Resistance Phenotypes of Uropathogenic *Escherichia coli* Isolates: A Retrospective Study

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Urinary tract infections caused by *Escherichia coli* are common causes of patients' referral to health centers and it is a common nosocomial infection that has recently become difficult to treat because of the increased emergence of drug-resistant strains. The present study aimed to determine frequency of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *E. coli* isolates originated from hospitalized patients.

### MATERIALS AND METHODS

Urine samples were obtained from 624 patients (age range  $\square$  35 years) in hospitals of Golestan province, northeast of Iran during 2018-2023. After identification of bacterial strains and isolation of *E. coli*, the Kirby-Bauer disk diffusion test was performed to evaluate the antibiotic susceptibility pattern to ten categories of antibacterial agents according to the Clinical and Laboratory Standards Institute M100 guidelines (2021). Minimum inhibitory concentrations (MICs) of drug-resistant isolates to gemifloxacin were determined using the microdilution broth method over a concentration range of 0.03-8  $\mu$ g/mL.

### RESULTS AND DISCUSSION

The frequency of isolates were *Escherichia coli* (49.50%), *Klebsiella pneumoniae* (22%), *Citrobacter freundii* (15.45%) and *Proteus mirabilis* (13.05%). Among *E. coli* isolates, the majority were from patients with urinary catheters (65.14%), most of which originated from ICU patients. The highest resistance and susceptibility among the *E. coli* isolates belonged to tetracycline (75.30%) and gemifloxacin (93.50%). Among *E. coli* isolates, 78.21% and 13.90% were MDR and XDR respectively. Moreover, Gemifloxacin (MIC=2  $\mu$ g/mL) inhibited the growth of 78% of XDR and 86% of MDR isolates (P=0.01).

### CONCLUSION

In this study, the high frequency of MDR/XDR phenotypes in the isolates may suggest an increasing trend of antibiotic resistance in *E. coli* strains. Our results indicated a high bactericidal activity of gemifloxacin against uropathogenic *E. coli* isolates. Considering the great inhibitory properties of gemifloxacin against MDR *E. coli* strains, it is suggested to investigate the efficacy of this agent for the treatment of urethritis particularly in patients with urinary catheters.

**Keywords:** *Escherichia coli*, Drug resistance, urethritis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating design of recombinant vaccine with fusion protein FimH, MrpH, FliC against urinary tract infections by proteus mirabilis: A review

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Urinary tract infection (UTI) is one of the most common infections in human societies and hospitals. *Proteus mirabilis* is known as one of the pathogens of urinary tract infection. The most common antigens of *Proteus mirabilis* are MrpH, FimH, and FliC. This study investigated the immunogenetics of fusion proteins MrpH, FimH, and FliC against urinary infections by *Proteus mirabilis*.

### MATERIALS AND METHODS

This study was a descriptive-analytical review that was using the search method and compilation of the original author's online articles in English published in PubMed, Science Direct, and Google Scholar databases. We have used keywords such as *Proteus mirabilis*, urinary tract infections, FimH, MrpH, and FliC.

### RESULTS AND DISCUSSION

Urinary tract infection is one of the most common causes of the pathogen *Proteus mirabilis* in different societies. Protein fusions as adjuvants and epitopes in vaccination were explored to prevent this infection caused by *P. mirabilis*. FimH, MrpH, and FliC proteins have been characterized by N-terminal domains. Activation of innate and adaptive immunity against UTI by the mentioned bacteria is possible through protein fusion through macrophage-lymphocyte interactions leading to the production of chemokines and cytokines. The design of a monovalent vaccine against it positive influence in preventing related pathogens has been studied, but more research is needed.

### CONCLUSION

In this study, with the help of a wide range of different strategic solutions for the fusion of tMrpH.FliC proteins to design a vaccine against urinary infections (UTIs) by *P. mirabilis*.

**Keywords:** *Proteus mirabilis*, urinary tract infections, FimH, MrpH, FliC

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation on the antimicrobial effects of nano-size starch biocomposite assimilated cumin essential oil (*Bunium persicum*) on *Salmonella typhimurium*, *Shigella flexneri* and *Klebsiella pneumonia* in vitro

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

In recent years, the use of herbal medicine, as a complementary and effective medicine, is increasing worldwide. Today, with the increasing resistance of bacteria to antibiotics, the use of medicinal plants has been welcomed. The aim of this study was to investigate the properties of antibacterial nano-size starch biocomposite assimilated Cumin essential oil in vitro.

### MATERIALS AND METHODS

In this experimental study, Cumin essential oil purchased from Zarin Golab Company (Kashan, Iran). The components of the essential oil were identified by gas chromatography and mass spectrometry (GC-MS). After preparing essential oil nanoemulsion, the particle size distribution and morphology of nanoemulsion were examined by Dynamic Light Scattering (DLS) and Transmission Electron Microscope (TEM) respectively, and then, its synthesized nano-capsules. Antimicrobial activity against *Salmonella typhimurium*, *Shigella flexneri* and *Klebsiella pneumoniae*, by disc diffusion, minimum inhibitory concentration (MIC), bactericidal (MBC) by microdilution methods and prevention of biofilm formation.

### RESULTS AND DISCUSSION

The result of BPEO analysis by GC/MS apparatus showed that the main of composition essential oil were Cumin aldehyde (30.40%), Phenylglycol (18.99%), *Y*-Terpinene (15.52%) The difference in chemical volatile compositions essential oil appertain to many factors such as, geographical conditions, soil conditions and else. The TEM and DLS gave parallel resulted. NCBP formulation were demonstrated had best effect than free essential oil and nanoemulsion of counterparts. The microbial cell membrane is damaged when the microorganisms are exposed to antimicrobial agents and the changes in the permeability of cell membrane could lead to the leakage of electrolytes from the interior of the bacterial cells

### CONCLUSION

With attention to the antibacterial properties of *Bunium persicum*, this compounds can be suggested in the treatment of bacterial infections.

**Keywords:** Nanoemulsion, Encapsulation, Cumin essential oil, Antibacterial activity, Gas Chromatography.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the bactericidal activity of a chimeric peptidoglycan hydrolase against *Staphylococcus aureus*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Antibiotics are one of the most important and influential medical inventions of the 20th century. However, the number of infections caused by multidrug-resistant (MDR) bacteria has been increasing since the beginning of the 21st century. *Staphylococcus aureus* is a pathogenic bacterium that is resistant to many antibiotics. With the increasing prevalence of antibiotic-resistant bacteria, phage endolysins are known as one of the promising alternatives to antibiotics. The desired chimeric endolysin has two catalytic domains and one domain binding to the cell wall, and they target peptidoglycan, causing cell lysis. The process of designing, cloning, and initial expression of this enzyme has been done by the Iranian Research Organization for Science and Technology (IROST). In this study, we demonstrated that desired endolysin has a high bactericidal activity against *Staphylococcus aureus*.

### MATERIALS AND METHODS

The desired plasmid is transformed into BL21 gold-competent cells through heat shock. Then protein expression was done for 24 hours at 15°C and finally, purification was done using HisPur™ Ni-NTA Resin by affinity chromatography and the protein concentration was determined. The purification was confirmed by the western blot analysis and the bactericidal activity of endolysin was evaluated by turbidity reduction assay (TRA).

### RESULTS AND DISCUSSION

Plate cultivation was done from the transformed BL21 gold and single colonies were observed in the plate containing TSA medium. Then the protein expression and purification were done and the desired 46 kDa protein band was observed in SDS-PAGE, and the purified protein band was approved by Western Blot. The protein concentration was measured with a BCA kit and the purified protein was 305 µg/ml. The bactericidal activity of endolysin was evaluated by turbidity reduction assay. Due to having two catalytic domains that target bacterial peptidoglycan, chimeric endolysin showed high bactericidal activity against *Staphylococcus aureus* and was effective even at a low concentration of 2.38 µg/ml.

### CONCLUSION

Antimicrobial drugs and new treatment options are needed to deal with antibiotic resistance. These results show that a new chimeric endolysin with higher activity and solubility can be created by domain swapping, which has great potential as an antimicrobial agent. Therefore, our strategy has significant potential for medical and biotechnological applications in combating multidrug-resistant bacteria such as *Staphylococcus aureus*.

**Keywords:** *Staphylococcus aureus*, multidrug-resistant, turbidity reduction assay, endolysin, Antimicrobial

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# "Exploring Yeast Protein as a Sustainable Alternative Protein Source for Nutrition, Climate Change, and Hunger Mitigation"

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The world population is growing rapidly and the demand for food is increasing, which poses major challenges to food security. The search for sustainable and healthier protein alternatives is a current focus of food research. In addition, yeast also contains protein biomass, trace elements, and vitamins including B groups.

This research investigates the potential of yeast protein as an alternative protein source derived from waste from various industrial sectors.

### MATERIAL AND METHODS

The study used a mixed-method approach, combining laboratory experiments and a systematic review of existing literature. The laboratory experiments involved the cultivation of yeast *Saccharomyces cerevisiae* on industrial medium containing molasses. The nutritional value of the yeast protein was evaluated through proximate analysis, and its amino acid profile was compared with that of conventional protein sources. The literature review focused on the environmental impact of traditional protein sources and the potential of yeast protein to reduce the ecological footprint of protein production.

### RESULTS AND DISCUSSION

The results showed that yeast protein from *Saccharomyces cerevisiae* has high protein content (50.0% and 55.0%, respectively) and also contains all essential amino acids in the required quantity according to FAO recommendation, making yeast SCP (single cell protein) a complete protein. The experimental data suggested that yeast protein could provide a viable alternative to traditional protein sources, such as soybean and animal-based protein which have higher environmental impacts. The literature review found that the production of yeast protein has a low carbon footprint and water usage, making it a sustainable protein source.

### CONCLUSION

In conclusion, this study highlights the potential of yeast protein as an alternative protein source to address hunger problems and mitigate the climate change. The study recommends the exploration for breeding high protein yeast strains, which are key indicators such as high growth rate on industrial waste.

**Keywords:** Yeast biomass, Alternative Protein Source, Nutrition, Climate Change

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Bacterial microflora of ruminants

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The microbial flora of the digestive system of ruminants includes microorganisms that have different enzymes to break down different plant polymers. In the rumen of ruminants, the microbial flora includes different groups: cellulolytic, lipolytic, pectinolytic, and amylolytic. The purpose of this article was to review articles written about ruminant microflora.

#### MATERIALS AND METHODS

In order to write and collect the contents of this study, various articles published in various prestigious magazines with similar positions have been used.

#### RESULTS AND DISCUSSION

Ruminants are herbivores that consume plant fiber as their main food source, but they do not have enzymes to digest it, ruminants have found a symbiotic relationship with microorganisms that have plant fiber-degrading enzymes. In the rumen of ruminants, the microbial flora includes different groups: a cellulolytic group such as *Ruminococcus albus*, lipolytic and pectinolytic groups such as *Treponema*, a proteolytic group such as *Perivotella*, an amylolytic group such as *Streptococcus bovis* and, an ureolytic group such as *Megasphaera elsednii*. Sometimes there is a disturbance in the uniformity of the microbial flora and it can cause complications such as bloating, rumen acidosis, hypoglycemia, diarrhea, ulcers in the gastrointestinal tract (GI).

The mentioned bacteria each play a role in breaking down different macromolecules. Therefore, the act of fermentation in the rumen of ruminants is also an example of their main tasks. In addition, it has been reported in many cases that microbial flora plays a significant role in increasing the quantity and quality of livestock products, including milk and meat. Also, some researchers are of the opinion that the fermentation of these microbes can prevent the growth of other harmful microbes.

#### CONCLUSION

According to the contents mentioned, the multiple roles of the microbial flora are quite evident, but sometimes these roles can be distorted due to various reasons, such as excessive nutrition and other secondary pollutions, and depending on the type of microbes, various complications can occur. Despite the various complications that may be caused by microbial flora, their practical roles in the digestive system are much more than their side effects.

**Keywords: Microflora, Ruminants, Rumen**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of the efficiency of Bacillol BODE solution in the elimination of microorganisms on the laboratory surfaces

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

A basic principle of disinfection is to use effective and safe disinfection solutions with minimal damage to equipment and staff. While none of the disinfectants are appropriate for all the various infectious agents and secondly, given that the choice of type of disinfectant is important in medical environments, there is a need for research to determine the effects of different disinfectants.

### MATERIALS AND METHODS

In this research, sampling took place at 30 different surfaces in a specialized medical diagnostic laboratory. Afterwards, all surfaces were disinfected with the commercial disinfectant solution Bacillol BODE and the sampling was redone. From samples collected before and after disinfection, microbial cultures were prepared on Mueller Hinton agar and colony counts were recorded using a colony counter. The data obtained were analyzed with the SPSS software and  $P < 0.05$  was considered a significant level.

### RESULTS AND DISCUSSION

THE results showed that in all the studied surfaces, after disinfection with Bacillol BODE solution, the number of colonies reached zero on average, which was significantly less than before disinfection ( $P < 0.05$ ).

### CONCLUSION

Bacillol BODE solution has a high ability to disinfect laboratory surfaces and was able to destroy all microbial colonies. Therefore, it can be used in both therapeutic and laboratory settings.

**Keywords:** Disinfectant Solution, Bacillol BODE, Laboratory Surfaces

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of the effect of zinc oxide nanoparticles prepared by green synthesis on *Pseudomonas aeruginosa* biofilm

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The ability of bacteria to build cellular assemblies on biotic and abiotic surfaces, immersed in a matrix of extracellular polymeric materials as a biofilm, has been demonstrated in recent years. This biofilm helps microbial populations in better nutrient uptake, protection from harmful circumstances, predation, desiccation, and exposure to antibacterial agents. It also plays a key role in the progression of bacterial pathogen disease. According to reports, bacteria that reside in biofilms are responsible for around 80% of bacterial infections. *Pseudomonas aeruginosa* is a pathogenic rod-shaped, Gram-negative bacterium that is widely distributed in water, plants, soil, and animals. It rarely causes infections in healthy people, but it is far more likely to do so in immune-compromised people. Previous investigation has indicated that nanotechnology is a viable strategy for treating infections caused by biofilms. There are many benefits to using nanoparticles (NPs) as drug delivery systems because they ensure continuous medication release and reduce unwanted side effects. A lot of research is also being done on the possible antibacterial properties of certain NPs. For instance, metallic oxide nanoparticles with minimal cytotoxicity and high drug delivery potential include zinc oxide (ZnO NPs) and copper oxide (CuO- NPs). It has been demonstrated that a variety of elements, including shape and surface charge, can significantly influence a nanomaterial's antibacterial and antibiofilm characteristics. In this study, we intend to reduce the biofilm formed by *Pseudomonas aeruginosa* by using a green synthesized nanoparticle.

### MATERIALS AND METHODS

To investigate the inhibitory effect of green synthesized nanoparticles on biofilm formation, we first prepared an overnight culture of the desired bacterium *Pseudomonas aeruginosa*. Then we prepared the 48-hour biofilm in 96-well plates. We fill the wells with a volume of 200µl of Mueller-Hinton broth (MHB). Then we prepare a bacterial suspension with a dilution of 10<sup>8</sup> CFU/mL and add 20µl to the wells containing the culture medium and incubate for 48 hours at 37°C. After this period, the wells were washed 3 times with phosphate buffer and then the same culture medium containing nanoparticles in the same initial volume was added to the wells. Then, it was re-incubated with the nanoparticle used in this research, which was prepared from orange peel by the researchers of Tabriz University Research Center, and to check the antimicrobial effect of nanoparticles, optical density at 600nm was measured by spectrophotometer. In this research, the MIC of nanoparticles was also prepared by the broth

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microdilution method. This was repeated in a 96-well plate, where 180µl of Mueller-Hinton broth containing bacteria at a concentration of  $2 \times 10^5$  CFU/mL was added to the first well, and 100µl of culture medium and bacteria were added to the other 5 wells. Then it was prepared from the initial concentration of zinc oxide nanoparticles (1000µg/ml) and serial dilution (500, 250, 125, 62.5, 31.25µg/ml). twenty µl of nanoparticles with a concentration of 1µg/ml were poured into the first well, then 100µl from the first well was pipetted into the second well, and this work was repeated until the fifth well, then incubated the plate at 37°C for 24 hours to check the minimum inhibitory concentration.

### RESULTS AND DISCUSSION

The morphological study of the green synthesized zinc oxide nanoparticles with orange was performed by SEM. The particles show a semispherical shape, and these particles are in a highly agglomerated form. The particle size ranged from 35.3 to 83.1 nm, respectively. The MIC results showed a weak antibacterial effect and growth inhibition occurred at a concentration of 500µg/ml. The results obtained from the spectrophotometer showed that the wells treated with nanoparticles compared to the control wells that only contained bacteria and were filled with empty medium after 48 hours, had a lower absorption number, and in the replicates, there was a 46% reduction in biofilm formed by *Pseudomonas* after 48 hours, so the nanoparticle prepared as a green synthesis was able to reduce the biofilm formed by *Pseudomonas aeruginosa*. In line with our study in 2014, Vincent et al. reported good anti-biofilm activity for zinc oxide nanoparticles in inhibiting the biofilm formation of strong biofilm-forming *Pseudomonas aeruginosa* bacteria at concentrations of 100, 200, and 500µg/ml, and the highest inhibition at the concentration they announced 500µg/ml. The results of the study by García-Lara and colleagues in 2015 showed that zinc oxide nanoparticles at a concentration of 1 millimolar have a strong inhibitory effect on biofilm formation by 26 to 100% in clinical and environmental strains of *Pseudomonas aeruginosa* and in the standard strain PAO1, at the rate of 94%.

### CONCLUSION

The results of the study showed that the biosynthesis of zinc oxide nanoparticles using orange peel without the need to spend energy and expensive raw materials is economically more economical than chemical and physical methods and is compatible with the environment and has many advantages. Such as the simplicity of the manufacturing method, good stability, less time consumption, non-toxic wastes, and large-scale synthesis capability.

**Keywords:** *Pseudomonas aeruginosa*, Biofilm, Nanoparticles, Green synthesis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Serological Detection of H9N2 Avian Influenza Virus Antibodies in Cats: Prevalence and predisposing Factors.

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The objective of this study was to investigate the prevalence and antibody titers of H9N2 avian influenza virus in cats. Specifically, the study aimed to determine the relationship between antibody titer and factors such as the type of food, place of storage, and contact with other animals. The findings of this research shed light on the potential transmission and prevalence of the H9N2 virus in cats, emphasizing the significance of monitoring and preventive measures.

### MATERIALS AND METHODS

Blood samples were collected from a total of 75 cats, including those referred to the veterinary clinic of Shahid Bahonar University in Kerman, private clinics, and stray cats in the city. Serum was separated from the blood samples and stored in a freezer. The hemagglutination inhibition (HI) test was conducted to detect antibodies against H9N2 influenza A virus. The HI test involved diluting the influenza antigen in a series of wells and mixing the suspected sera from the cats with washed red blood cells and the antigen. The test plates were observed for hemagglutination, and the highest dilution exhibiting complete erythrocyte sedimentation was considered as the serum antibody titer.

### RESULTS AND DISCUSSION

Out of the 75 tested samples, 49 (65%) displayed a positive antibody titer against the H9N2 influenza type A virus. Statistical analysis revealed significant associations between the antibody titer and factors such as the type of food (raw or cooked), place of storage (inside or outside the house), and contact with other animals. Cats fed raw food exhibited a higher positive titer (67.83%) compared to those fed cooked food (7.30%). Similarly, cats kept outside the house demonstrated a higher positive titer (5.79%) than those kept inside (4.38%). Moreover, cats with contact with other animals had a higher positive titer (1.74%) compared to those without contact (2.35%). These results indicate a potential prevalence of H9N2 influenza type A virus in cats. Furthermore, they highlight the influence of various factors such as diet, environment, and animal contact on the transmission and spread of the virus among cats. The higher positive titer observed in cats fed

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raw food suggests a possible role of diet in virus exposure. Similarly, cats kept outside the house and those with contact with other animals are more likely to come into contact with the virus, leading to higher antibody titers. These findings underscore the importance of considering these factors in strategies aimed at monitoring and preventing the transmission of H9N2 influenza type A virus among cats.

### CONCLUSION

The study demonstrated a notable prevalence of H9N2 influenza type A virus antibody in cats, indicating their potential susceptibility to the virus. The results reveals that significant factors such as diet, environment, and contact with other animals can influence the transmission of the virus among cats. To mitigate the risk of infection with H9N2 virus, proper dietary management, restricted contact with contaminated animals and better housing conditions should be implemented.

**Keywords: H9N2 Avian Influenza, Cats, Antibody Titers, Prevalence, Transmission, Diet, Environment, Animal Contact, Monitoring, Preventive Measures**

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# Seroprevalence study of Avian H9N2 Influenza Virus in Dogs: A Zoonotic Risk Assessment in Kerman, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Influenza is a highly contagious zoonotic disease caused by influenza viruses, with avian H9N2 influenza virus being a significant pandemic pathogen worldwide. Considering the risk of transmission from pets to humans, this study aims to assess the prevalence of antibodies against avian H9N2 influenza virus in dogs in Kerman, southeast of Iran.

### MATERIALS AND METHODS

Serum samples were collected from 170 healthy dogs in Kerman from September 2012 to February 2013. A hemagglutination inhibition assay was used to evaluate the presence of antibodies against avian H9N2 influenza virus. Data on age, gender, diet, housing type, and contact with other animals were collected through a questionnaire.

### RESULTS AND DISCUSSION

Out of 170 samples, 65 (38.23%) tested positive for H9N2 antibodies. Dogs with raw diets and those kept in farms with other animals showed higher antibody levels. The susceptibility of dogs to avian H9N2 virus has been previously reported, and they can act as potential sources for interspecies transmission and the creation of reassortant influenza viruses. However, the epidemiology and distribution of avian H9N2 influenza virus in dogs, particularly in the southeast region of Iran, had not been previously studied.

### CONCLUSION

The study highlights the significant seroprevalence of avian H9N2 influenza virus in dogs in Kerman, Iran. The findings emphasize the importance of monitoring and controlling infection transmission from dogs, especially in regions with widespread H9N2 distribution. To reduce the infection burden and zoonotic risks, close attention to farm dogs and dogs which fed with raw diets seems vital. Public health authorities should consider the role of pets, such as dogs, in transmission of influenza viruses, control achievement and preventive programs respectively.

**Keywords:** Avian H9N2 influenza virus, Dog, Hemagglutination inhibition, Iran, Seroprevalence

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# Diet, Genetics and Probiotics: The Key Factors for a Healthy Gut Microbiome

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

In this study we explain that the gut microbiome is the genetic material of the microorganisms that live in the human digestive system and that dysbiosis, or an imbalance of gut bacteria, has been shown to be closely associated with certain diseases and condition.

### MATERIALS AND METHODS

This study is based on a literature search and analysis of the existing studies on how diet, genetics, and personalized approaches for probiotics affect the microbiome and its detoxification capacity. The articles were identified from English databases using keywords.

### RESULTS AND DISCUSSION

The microbiota and the anti-inflammatory molecules produced by bacteria can be affected by diet. Consuming fiber, protein, fat, polyphenols, tryptophan, and probiotics, prebiotics and supplements can modification these molecules. The gut microbiome composition can be affected by genetics, which determine which microbes can live and grow in the gut and how they interact with the host factors. Different genetic variants can affect the intestinal mucosal barrier, the immune response, and the drug and food metabolism of the host, which in turn can affect the availability of nutrients, oxygen, and other factors for the microbes. Some genetic variants can also affect the susceptibility and response to certain diseases or conditions that are related to these microorganisms. Different genetic variants and the gut microbiome interact and affect each other like LCT-MCM6 (Bifidobacterium and lactose), ABO-FUT2 (Faecalicatena lactaris and blood antigens), MED13L (Enterococcus faecalis), NOD2 (Roseburia, Eubacterium rectale and butyrate), TNFSF15 (Ruminococcus gnavus and IBD), SLC23A1 (Akkermansia muciniphila, mucin, obesity and metabolic syndrome). Personalized approaches for probiotics aim to identify the most suitable probiotic strains and doses for each individual, based on their specific characteristics and goals. Using microbiome data, host data, feedback data such as self-reporting can help to optimize the benefits of probiotics for detoxification by reducing the variability and uncertainty of the probiotic effects.

### CONCLUSION

Diet, genetics, and personalized approaches for probiotics can affect the metabolism and balance of the microbiome and its detoxification capacity and prevent negative consequences for our nutrition genetics, and disease. Therefore, it is necessary to understand how these factors influence the microbiome and how to modulate it for optimal detoxification.

**Keywords: Dysbiosis, Microbiota, Probiotics, Fermented Foods, Genetic**

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# Investigating the resistance to glycopeptide and aminoglycoside antibiotics in *Enterococcus* species isolated from the feces of domestic animals around Rasht City

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Enterococcus*, the natural intestinal flora of humans and many animals, is the second cause of infection in hospitals and the third most common cause of hospital bacteremia. Resistant enterococci are found almost everywhere; they can be transferred to healthy people in society and spread in the non-human community. Animals, as the large reservoir of resistance genes, may transfer these genes to saprophytic bacteria or even dangerous pathogens. The present study aimed to identify and determine the resistance to glycopeptide and aminoglycoside antibiotics in enterococci isolated from the feces of domestic animals around Rasht city.

### MATERIALS AND METHODS

*Enterococcus* isolates were segregated from 80 horse and cattle feces samples around Rasht city, and the culture and biochemical tests were used to identify them. The antibiotic resistance pattern was investigated by the disk diffusion method, the minimum inhibitory concentration of bacteria (MIC) using the macrodilution broth method, and the frequency of resistance genes by PCR method in the isolates.

### RESULTS AND DISCUSSION

Out of 30 cattle and 50 horse isolates, the highest resistance in both groups to ampicillin was 100% and 96%, respectively. Tetracycline and enrofloxacin were the most effective antibiotic in horse and cattle isolates, respectively. In 4 horse and 2 cattle isolates with vancomycin MIC > 32 µg/ml, none of the *vanA* or *vanB* genes were detected, also in 38 horse and 19 cattle isolates resistant to the aminoglycoside gentamicin and amikacin, the frequency of *aac(6')-aph(2'')* and *aph(3')-IIIa* were 55/26%, 73/68%, 76/31%, and 63/16%, respectively.

### CONCLUSION

The results indicate the resistance of enterococcal animal isolates to the studied antibiotics, the presence of high-level resistance to aminoglycosides, and the presence of aminoglycoside resistance genes in these isolates. The spread of these strains in the environment can threaten livestock owners and public health.

**Keywords:** *Enterococcus*, Domestic Animals, Antibiotic Resistance, Resistance Gene

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### ***Chlamydia abortus* Detection in Post-Partum Secretions of Iranian Lori Breed Ewes**

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#### **ABSTRACT**

#### **BACKGROUND AND ABJECTIVE**

*Chlamydia abortus* is an intracellular gram-negative pathogen that has been reported as one of the main causes of abortion in sheep in many countries of the Middle East, causing enzootic abortion of ewe (EAE). Bacterium pose a zoonotic risk to workers, farmers, and veterinarians as well. In Human mild influenza-like symptoms can be brought on by ingesting contaminated food or inhaling infective droplets. In sheep the disease that results from *C. abortus*' initial invasion of mucosal membranes and subsequent genital system incursion manifests as abortion, particularly in the final two to three weeks of pregnancy, stillbirth, or lambing that appear to be healthy but are actually infected. Pneumonia, enteritis, encephalomyelitis, conjunctivitis, seminal vasculitis, and orchitis are additional clinical signs that frequently need to be treated in infected sheep. Before gestation, infected ewe does not exhibit any clinical symptoms of infection. The main sources of infection spread among susceptible flocks are genital secretions prior to abortion or during parturition, as well as the products of abortion including infected placentae, fetuses, and the coats of neonates. The most specific method for diagnosing EAE includes isolating the bacterium or DNA amplification from an abortive sample or vaginal discharge by molecular methods such as PCR, identifying the outer membrane genes (*ompA*). EAE has frequently been studied serologically reported in sheep in Iran. The purpose of this study is to look into whether *C. abortus* was present in the post-partum secretions of Lori breed sheep that had a full-term delivery. by using the real-time PCR method.

#### **MATERIALS AND METHODS**

Sampling was done from the post-partum secretions of 36 Lori ewes that had full-term and healthy lambing (by using swabs). These animals have a history of abortions. A cold chain system was used to transport the gathered samples to the lab after they had been stored in sucrose-phosphate glutamate (SPG) buffer. Until further use, the samples were kept at -20 °C. The High Pure PCR Template Preparation Kit (Roche Company, Germany) was used to extract the DNA from samples in accordance with the manufacturer's instructions. The DNA was stored at -20 °C pending analysis. Taq-man real-time PCR was used to amplify the *ompA* gene in order to qualitatively detect *C. abortus*. Cincloon Co. (Iran) primers and probes of the target genes were employed. The TaqMan Universal PCR Master Mix (1×), 1 µl of DNA sample, 10 pmol primers, 5 pmol probe, and 5 ng of IC template were used to perform the final 25 µl reaction mixtures. On Rotor Gene Q

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(QIAGEN Marseille S.A.), amplification was performed using the following temperature schedule: preheat at 95 °C for 10 minutes, followed by 40 two-step cycles at 15 and 60 degrees Celsius for 60 seconds. The cycle threshold (Ct value) was calculated automatically.

### RESULTS AND DISCUSSION

According to real-time PCR results, sixteen samples tested positive for *C. abortus*. The fact that *C. abortus* was positive in postpartum secretions in ewes who had a normal delivery with healthy lambs is crucial. The presence of *C. abortus* in samples obtained from ewes following parturition has been investigated in studies, including Livingstone et al.'s research in 2009. They discovered *C. abortus* DNA in placentas from ten ewes with a history of abortion. The ewes under study have healthy lamb in their subsequent lambing. They used the real-time PCR technique.

In 2007 a British study reported 30.9% prevalence of *C. abortus* DNA among uterine tissue of three hundred and four ewes at an abattoir by conventional PCR. In 2018 Barkallah et al. detected *C. abortus* by PCR method in 8.7% of swabs from sheep in Tunisian and as described by Merdja et al., 13 out of 199 (6.5%) swabs were positive for *C. abortus* by Real-time PCR in Algeriam in 2015. Four out of the eleven fetal membrane samples collected in a 2011 study by Gutierrez et al. contained low numbers of *C. abortus* in subsequent parturition with EAE history. They claimed that low levels of ewes infection after primary infection can cause them to remain chronically or persistently infected. In the aforementioned study, no animals aborted in the second lambing season and the length of gestation was not different between pregnancy outcomes. Ewes are considered to be naturally infected with *C. abortus* via the oral–nasal route and may become persistent carriers, shedding during subsequent oestrous cycles and at lambing. According to studies, ewes with a history of EAE are thought to be immune to further lamb loss after having an abortion. In the case of present study, ewes with a possible EAE history all have full term delivering during subsequent pregnancy.

### CONCLUSION

The findings of the current study demonstrate that *C. abortus* can still be excreted in post-partum secretion even after healthy, normal lambing. This issue emphasizes the possibility that the infection could spread among the herd if it is not properly managed. Infectious elementary bodies are shed into the environment and may be inhaled by exposed animals. This issue will become more important since vaginal discharge can last up to three weeks after lambing. A characteristic feature of EAE is that ewes which abort following infection do not abort again in subsequent years. In the current study, it's possible that *C. abortus* was the cause of the previous ewes' abortion. The findings of the current study, along with the high rate of EAE in various Iranian regions, economic losses, and risk of human infection, call for extra focus in order to advance research and put preventive and control measures into practice. According to research like Esmaili et al. 's 2021 study, Small ruminant herds in Iran had a high prevalence of *C. abortus*, so vaccination is strongly advised for Iranian flocks. To lessen the adverse effects, ranchers must receive training and education in quarantine and sanitary practices. The implementation of a successful control program requires an understanding of epidemiologic features. It is advised to conduct additional research on different animal species to identify the role that *C. abortus* and other agents play in small ruminant abortions.

**Keywords:** Lori breed, *Chlamydia abortus*, Lambing, Real-time PCR, Ewes, Enzootic Abortion of Ewe (EAE)

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# Investigating the *Coxiella burnetii* Presence in Healthy Placenta of Goats with Full-Term Delivery

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

In Iran, goat farming is still a common custom. These animals are kept to production and consume raw materials like milk, meat and wool. A producer's economic situation and a concern for health and food safety are both affected by abortions. The goat industry suffers significant financial losses as a result of abortion. One of the infectious agents with the highest potential for abortion is *Coxiella burnetii*, witch a significant zoonotic agent that is widely prevalent worldwide. Birth products, vaginal secretions, milk, and the feces of infected domestic ruminants are the main sources of *C. burnetii*. This obligate intracellular pathogen is highly tenacious and resistant to numerous disinfectants, heat, UV light, and desiccation. The effects of *C. burnetii* on goat health include weak kids, stillbirth, and high rates of abortion in goats. The current study concentrated on the *C. burnetii* presence in full-term placenta from healthy goats.

### MATERIALS AND METHODS

In present study, forty-three pregnant Saanen goats who delivered at full term were examined. These animals didn't exhibit any clinical signs of *C. burnetii* infection, nor did their placentas or labor secretions seem to be having any issues. There was no history of abortion among the chosen animals. As soon as the placenta was discharged, a sample was taken. In the shortest amount of time possible, the samples were delivered to the laboratory. In samples, *C. burnetii* DNA was detected using PCR. DNA was extracted from the vaginal samples using DNA extraction kit (CinnaGen- Iran) according to the manufacturer's instructions. Forward and reverse primers were used to amplify a fragment of the *C. burnetii* IS1111 gene that was 687-bp, including TATGTATCCACCGTAGCCAGTC and CCAACAACACCTCCTTATTC were used respectively.

### RESULTS AND DISCUSSION

*C. burnetii* DNA was found in the placenta of forty goats, according to PCR results. And after full-term delivery, these goats shed coxella. This is entirely consistent with the field observations that showed a goat's placenta can contain *C. burnetii* when it gives birth normally. In 2012, Rosette et al. inoculated pregnant goats with *C. burnetii* via the intranasal route. observed that newborn kids from infected goats had high amounts of *C. burnetii* that were

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excreted with the placenta. Importantly, since infected goats gave birth to both live and dead kids, *C. burnetii* infection does not always result in abortion. According to studies, healthy excreting goats are most likely to be responsible for the long-term spread of *C. burnetii* in a herd. This assertion is supported by the findings of the current study. placental isolates of *C. burnetii* have also been shown to be more virulent than other isolates. the majority of studies carried out in Iran concentrated on the bacterium that sheds through milk and its risk factors for humans. in such a way that other methods of shedding, like placental route, were disregarded. The current study demonstrated that placenta is a significant contributor to environmental contamination and bacterial shedding. It is suggested that research could be done on the fate of infants born to healthy goats who shed *C. burnetii*.

### CONCLUSION

The positive PCR results probably indicates the occurrence of a latent infection that may have remained in the localized to the placenta. In this situation, at the time of delivery and peripartum, there is likely bacterial excretion in the amniotic fluid and vaginal mucus, which contaminate the environment. Studies also point out that during abortion and delivery, infected pregnant goats primarily excrete *C. burnetii*. Environmental contamination from such excretions raises the possibility of infection to other vulnerable goats in the herd because these bacteria can survive for extended periods of time. Accordingly, when Q fever appears in a goat herd, the controlling procedures should be maintained for a long periods of time. This pathogen's Excretion should be regarded as a major zoonotic risk to humans. The present results support the need to continue the Q fever public information, particularly among those who are occupationally exposed, and highlight the significance of this disease in monitoring and preventing programs for public and livestock health. According to the current findings and other studies of a similar nature, special attention to asymptotically infected goats and vaccination, which has been proven to be one of the most effective methods for lowering bacterium shedding and the incidence of abortion, are important strategies.

**Keywords:** Placenta, *Coxiella burnetii*, Abortion, PCR, Full-Term Delivery, Q fever, Saanen

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# Prevalence of Biofilm-Related Genes and Biofilm Forming Ability among Clinical Isolates of *Pseudomonas aeruginosa*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa* is an opportunistic pathogen in the health-care system and a leading causative agent of high mortality in hospitalized patients significantly, immunocompromised. Biofilm formation system involves expression of various virulence factors and development of drug resistance that causes prolonged patient infections. This study was performed to determine the antibiotic resistance pattern, biofilm formation and frequency of biofilm-related genes in *P. aeruginosa* strains.

### MATERIALS AND METHODS

A total of 123 isolates of *P. aeruginosa* were collected from different clinical specimens. Antimicrobial susceptibility testing (AST) was performed to detection of Multidrug resistant *P. aeruginosa* (MDRPA) isolates. In order to evaluate the biofilm-forming isolates, microtiter plate (MTP) method was carried out. Also, the prevalence of biofilm genotype patterns (*PslA*, *PslD*, *PelA*, *PelF* and *AlgD*) was detected by Polymerases Chain Reaction (PCR).

### RESULTS AND DISCUSSION

According to our findings, the most resistance and susceptibility rates were found in ceftazidime with 74.7% and ciprofloxacin 42.2%, respectively. Also, wound isolates had the highest level of resistance and meropenem were the most active antimicrobial agent against them. In total, 86.1% *P. aeruginosa* isolates were found as MDRPA, of which 61.3% were able to form strong biofilm. The highest and lowest frequency of biofilm-related genes among biofilm producer isolates belonged to *PelF* 82.1% and *AlgD* 55.2%, respectively.

### CONCLUSION

The findings of the conducted study indicate a significant relationship between MDRPA and biofilm genotypic/phenotypic patterns, and suggest the necessity of careful surveillance program in hospital settings. Administration of anti-biofilm agents could be an alternative approach for eradicating of resistant isolates, which are life-threatening pathogens worldwide.

**Keywords:** *Pseudomonas aeruginosa*, Antibiotic resistance, Biofilm-related gene, Nosocomial infections

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of Quorum-Sensing System Genes and Drug Resistance among Clinical Isolates of *Pseudomonas aeruginosa*, in Hamadan, West of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa* is an opportunistic pathogen in the health-care system and a leading causative agent of high mortality in hospitalized patients significantly, immunocompromised. Quorum sensing (QS) system involves biofilm formation, expression of various virulence factors, and development of drug resistance that causes prolonged patient infections. Therefore, due to the significant role of the QS system in enhancing the pathogenicity of *P. aeruginosa*, the primary objective of this study was to evaluate the prevalence of QS genes, as well as the biofilm-forming capacity and antibiotic resistance pattern among *P. aeruginosa* strains.

### MATERIALS AND METHODS

A total of 120 isolates of *P. aeruginosa* were collected from different clinical specimens. The disk diffusion method was performed to detect the antibiotic resistance pattern and MDRPA/XDRPA strains. The microtiter plate (MTP) method was applied to investigate the biofilm-forming ability of isolates. Finally, the prevalence of *rhII*, *rhIR*, *lasI*, and *lasR* genes was assessed by the polymerase chain reaction (PCR) method.

### RESULTS AND DISCUSSION

Overall, 106 (88.3%) and 32 (26.6%) isolates were found as MDRPA and XDRPA, respectively. The most and the less resistance rates were shown against ceftazidime (75.0%) and ciprofloxacin (46.6%), respectively. The prevalence rates of QS genes among all investigated isolates were as follows: *rhII* (81.6%), *rhIR* (90.8%), *lasI* (89.1%), and *lasR* (78.3%). The most common type of QS genes among MDRPA isolates were related to *rhIR* and *lasI* with 94.3%. Also, *rhII*, *rhIR*, and *lasI* genes were positive for all XDRPA isolates. Also, *rhIR* (94.7%) and *lasR* (81.7%) genes have the highest and lowest frequency among biofilm-forming isolates, respectively.

### CONCLUSION

Our findings revealed the significance of the QS system in biofilm formation and development of MDRP/XDRPA strains. Administration of QS inhibitors could be a promising strategy for preventing the spread of resistant isolates, which are life-threatening agents worldwide.

**Keywords:** *Pseudomonas aeruginosa*, Quorum sensing, Biofilm formation, Drug-resistant

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Prevalence of *Mycobacterium tuberculosis* mutations associated with isoniazid and rifampicin resistance: A systematic review and meta-analysis

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#### ABSTRACT

Tuberculosis (TB) is still one of the leading causes of worldwide death, especially following the emergence of strains resistant to isoniazid (INH) and rifampicin (RIF). This study aimed to systematically review published articles focusing on the prevalence of INH and/or RIF resistance-associated mutations of *Mycobacterium tuberculosis* isolates in recent years. Literature databases were searched using appropriate keywords. The data of the included studies were extracted and used for a random-effects model meta-analysis. Of the initial 1442 studies, 29 were finally eligible to be included in the review.

The overall resistance to INH and RIF was about 17.2% and 7.3%, respectively. There was no difference between the frequency of INH and RIF resistance using different phenotypic or genotypic methods. The INH and/or RIF resistance was higher in Asia. The S315T mutation in KatG (23.7 %), C-15T in InhA (10.7 %), and S531L in RpoB (13.5 %) were the most prevalent mutations. Altogether, the results showed that due to S531L in RpoB, S315T in KatG, and C-15T in InhA mutations INH- and RIF-resistant *M. tuberculosis* isolates were widely distributed. Thus, it would be diagnostically and epidemiologically beneficial to track these gene mutations among resistant isolates.

**Keywords:** Antibiotic resistance, Isoniazid, Rifampicin, mutation, *Mycobacterium tuberculosis*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Encapsulation of rOmpA from *Klebsiella pneumoniae* in Silk Fibroin-Sodium Alginate Nanoparticles: A New Approach to Developing a Pneumonia Vaccine

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

The present study describes the design and fabrication of a new vaccine candidate based on the outer membrane protein A (rOmpA) from *Klebsiella pneumoniae* (*K. pneumoniae*) encapsulated in silk fibroin-sodium alginate nanoparticles (SF-SANPs) against *K. pneumoniae*-mediated pneumoniae.

### MATERIALS AND METHODS

The physicochemical properties, toxicity, release profile, and *in vivo* potency of SF-SANPs encapsulated with rOmpA were evaluated.

### RESULTS AND DISCUSSION

The spherical nano vaccine was created with an average particle size of 160 nm and an encapsulation efficiency of 80%. Antigen release from SF-SANPs was 40% after 22 days release assay. The SF-SANPs showed a zeta potential of -24.8mV and had no toxic effect on the L929 cells *in vitro*. The study also found that SF-SANPs in the vaccine formulations promoted systemic (IgA, total IgG, IgG1 (Th2), and IgG2a (Th1)) and mucosal (IgA and IgG) antibodies and stimulated cytokine response (IFN- $\gamma$ , IL-4, and IL-17), inducing both humoral (Th2) and cell-mediated (Th1) immune responses, with a Th1-polarized response.

### CONCLUSION

The vaccine was effective in protecting the lung against experimental pneumoniae and reducing inflammation. These findings suggest that the rOmpA-based vaccine encapsulated in SF-SANPs could be a promising strategy for preventing pneumonia caused by *K. pneumoniae*.

**Keywords:** Silk fibroin-sodium alginate nanoparticles, Nanoadjuvant, Vaccine, Pneumoniae, *Klebsiella pneumoniae*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and identification of mycobacteria isolated from raw milk in Tehran based on 16srRNA and hsp65 genes

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

As an important source of human food, milk can be a carrier of human pathogenic bacteria, including tuberculous and nontuberculous mycobacteria (NTM), in its raw and unpasteurized state.

#### MATERIALS AND METHODS

In this research, 175 raw milk samples were collected from traditional dairy stores in 22 regions of Tehran in a 9- month period from August 2019 to May 2020. Samples were prepared and transferred to a specialized laboratory, where they were inoculated in Lowenstein-Jensen (LJ) medium containing glycerol or sodium pyruvate, as well as Herrold's egg-yolk with and without Mycobactin J. to determine the sample's identity of samples. The recommended 16S rRNA (1436 bp) and hsp65 (644 bp) genes fragments from the positive isolates identified in Ziehl-Neelsen (Z-N) staining were amplified and sequenced using PCR and compared with the sequences of the gene fragments of reference strains available in the global GenBank database 16S rRNA SILVA, 16S rRNA RDP and nBLAST NCBI. ...

#### RESULTS AND DISCUSSION

In case of samples, a total of four bacteria were collected, all of which were found in the genetic differential testing to be NTM, including n=1 *Mycobacterium heraklionense*, n=2 *Mycolicibacterium fortuitum*, and n=1 *Mycobacterium thermoresistibile*.

#### CONCLUSION

The analysis of the results obtained by isolates sequencing using the 16S rRNA gene showed higher discriminatory power and percentage similarities in the identification of the isolates than the hsp65 gene.

**Keywords:** *Mycobacterium*, *Mycolicibacterium*. Raw milk. 16S rRNA gene. hsp65 gene

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Gastrointestinal microbiota as a double-edged sword for gastrointestinal cancer

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

In an attempt to determine the etiology of carcinogenesis and potential prognostic indicators, researchers have always been interested in the relationship between microorganisms and gastrointestinal (GI) carcinogenesis. There is ample evidence to suggest that the GI microbiota, specifically the pro-inflammatory and immunosuppressive signals it produces, contributes to the development of cancer.

Microbiota profiles vary widely within and between populations, depending on factors such as age, diet, lifestyle, genetics, antibiotic use, and environmental factors. As a result, it is more appropriate to examine the GI tract microbiome in carcinogenesis. In the case of gastrointestinal cancers, examining the host's microbial profile may be necessary to gain important insight into the disease state. A better understanding of GI cancer and the host microbiota is necessary to gain important insights into the disease state.

This review focuses on the role of the microbiota in the development of gastrointestinal cancers. We discuss how microbiota and their metabolites can increase the risk of cancer.

### MATERIALS AND METHODS

Numerous databases were employed for this investigation, including Google Scholar, PubMed, EMBASE, and Scopus, each of which contained a large number of publications.

Keywords like "Gut microbiome", "Gastrointestinal microbiota" and "Gastrointestinal cancer" were also utilized.

### RESULTS AND DISCUSSION

The microbiota of the gastrointestinal tract, including the microbiota of the mouth, intestines, and stomach, through existing mechanisms, can predispose people to cancer or act as a cancer prevention factor. Just like a double-edged sword. Based on our findings, several intestinal bacteria such as *Clostridium butyricum*, *Lactobacillus*, *Enterococcus faecalis*, *Bacteroides fragilis*, and *Bifidobacterium bifidum* have therapeutic potential against gastrointestinal cancers using different mechanisms.

### CONCLUSION

Considering the different roles of microbiota, both in causing cancer and in therapeutic potential, it is important to know more and better the microbiota of the gastrointestinal tract and their mechanism of action. Therefore, in this review, we try to know more about these factors.

**Keywords:** Gut microbiome, Gastrointestinal microbiota, Gastrointestinal cancer

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The occurrence of multidrug-resistant *Mycobacterium tuberculosis* from suspected patients of tuberculosis in Ilam, Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) is one of the leading causes of death in the world. The resource constraints make it difficult to diagnose and monitor the cases of MDR-TB. GeneXpert is a recognized tool used to diagnose the patients of pulmonary tuberculosis in clinical settings across the globe.

#### MATERIALS AND METHODS

A hospital-based cross-sectional study was conducted among 61 tuberculosis suspected patients from 21 March to February 2022. Laboratory examination was processed using Xpert-MTB/RIF assay.

#### RESULTS AND DISCUSSION

In this study, the prevalence of *Mycobacterium tuberculosis* was 6 (10.1%), and there was no case of rifampicin-resistant/multidrug-resistant *Mycobacterium tuberculosis* among the 6 confirmed cases of *Mycobacterium tuberculosis*.

#### CONCLUSION

Despite implementation of national and international TB control strategies, TB still remains one of the major public health problems. Our data suggests that the prevalence of drug resistant TB remains low in Ilam, and Xpert MTB/RIF assay appears to be an accurate, simple, and useful technique for detecting MTB, especially in respiratory specimens.

**Keywords:** Xpert-MTB/RIF assay, *Mycobacterium tuberculosis*, tuberculosis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Identification of *Mycobacterium tuberculosis* complex from suspected patients of tuberculosis by conventional and molecular methods in Ilam, Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

TB is an airborne disease caused by *Mycobacterium tuberculosis* (MTB). The bacterium mostly infects the lungs, but many other tissues or organs can be affected. Tuberculosis (TB) kills approximately three people every minute. According to estimates, 10.6 million people fell ill with TB in 2020 and 1.6 million died from the disease in 2021. More than 90% of new TB cases and deaths occurring in low and middle-income countries. Poverty and poor hygiene habits can weaken the immune system and raise the risk of TB. The present study was performed aiming to isolation, identification and prevalence of *Mycobacterium tuberculosis* in cases of suspected TB.

#### MATERIALS AND METHODS

In total, 591 suspected cases of tuberculosis were investigated from March 21, 2022 to March 21, 2023. Genomic material was extracted from all acid-fast positive cultures. The mycobacterial identity of bacterial isolates was confirmed using a PCR assessment.

#### RESULTS AND DISCUSSION

A total of 6 isolates were obtained from 591 cultures submitted to the laboratory of Tuberculosis Department Razi Vaccine & Serum Research Institute, Karaj, Iran. Species identification was performed for all isolates using conventional phenotypic methods and PCR assessment targeting a 543 bp- fragment of 16Sr RNA gene, 245 bp- fragment of IS6110 and RD typing.

#### CONCLUSION

In recent decades, various genotyping and phenotyping strategies have been used to distinguish and identify mycobacterium species. Spoligotyping and RD typing methods are widely used to investigate the epidemiology of *Mycobacterium tuberculosis* complex.

To conclude, in this region the prevalence of tuberculosis is lower than the global and national average. It seems that more studies like MIRU-VNTR and Spoligotyping are required to provide a reliable biogeographical map of TB in this province and Iran.

**Keywords:** *Mycobacterium tuberculosis*, PCR- IS6110, PCR- 16SrRNA, RD typing

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and identification of mycobacteria in aquarium fish in Ilam, Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Fish, like mammals, are susceptible to mycobacteria, and can act as both host and carrier for these bacteria. Mycobacteriosis is a chronic progressive disease, which have been reported in more than 200 species of fish. Although *M. marinum*, *M. chelonae* and *M. fortuitum* are the most common causes of infections in fish. These mycobacteria are saprophytes found in soil and water. Mycobacteriosis is a zoonotic and occupationally related disease capable of causing local and disseminated infections in humans. The number of *M. fortuitum* infections in humans is increasing.

#### MATERIALS AND METHODS

In total, 50 different samples of ornamental fish were collected from different aquariums in Ilam. Genomic material was extracted from all acid-fast positive cultures. The mycobacterial identity of bacterial isolates was confirmed using a PCR assessment.

#### RESULTS AND DISCUSSION

A total of 12 NTM isolates were obtained from 50 cultures submitted the laboratory of Tuberculosis Department Razi Vaccine & Serum Research Institute, Karaj, Iran. Species identification was performed for all isolates using conventional phenotypic methods and PCR assessment targeting a 543 bp- fragment 16Sr RNA gene and 441 bp- fragment of *hsp65*.

#### CONCLUSION

The current study clearly demonstrates the role of mycobacteria in the pathogenesis of diseases in ornamental fish. People who keep fish as pets at home should be aware of the dangers of bacterial contamination risks arise by close contact with ornamental fish species. The results we obtained indicated that species identification by molecular methods was more reliable and precise than the phenotypic methods, as previously reported by other investigators.

**Keywords:** Ornamental fishes, Mycobacteriosis, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The role of microbiota in health and disease

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Maintaining physical and mental health has always been a major challenge in medicine since genetics, environmental factors, mobility, and chronic diseases have altered human health. In addition to understanding how the microbiota changes over a person's lifetime, understanding how it affects health and disease is also crucial. Heart disease, obesity, diabetes, mental disorders, neurological disorders and kidney disease are all affected by the gut microbiota.

#### MATERIALS AND METHODS

Today's lifestyle is characterized by inactivity, high-calorie foods, and excessive antibiotic use, all of which are closely related to the gut microbiome, suggesting that intestinal microbiota play an important role in chronic disease. Gut microbiota is dominated by (Firmicutes and Bacteroides) 80%, actinobacteria less than 10%, and proteobacteria less than 1%. A bacterial LPS response induces inflammation and stimulates immune cell infiltration. The breakdown of fat-laden foods produces TMAO metabolites that increase cholesterol levels and cause cardiovascular disease. This mechanism of communication between gut microbiota and liver, brain, and adipose tissue regulates fatty synthesis. In addition, SCFAs inhibit lipolysis by activating GPR43 and GPR41 receptors and contribute to fat accumulation in fatty cells by promoting insulin-mediated fat accumulation. With quantitative real-time PCR, it is possible to evaluate gut microbiome composition at low cost. Other methods for determining the sequence and targeting the V4-V5 region of the 16S rRNA gene.

#### RESULTS AND DISCUSSION

The consumption of high-calorie foods, fats or proteins can alter the gut microbiome, resulting in harmful metabolites being produced and disease developing. By consuming fiber, fruits and vegetables, the microbiome changes to reduce harmful metabolites. It is imperative that intestinal homeostasis and dysbiosis are maintained by beneficial intestinal bacteria, such as those that produce short-chain fatty acids or endotoxins.

#### CONCLUSION

In today's world, the microbiota plays an important role in the prevention, care, and treatment of diseases. We hope that with more research in this area, chronic diseases will be reduced.

**Keywords: Gut Microbiota, Dysbiosis, Microbiome, Intestinal Microbiota**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Design, Synthesis, and Antimicrobial Evaluation of N-(2,5-Dihydro-pyrimidine-2-yl)-4-methyl-benzenesulfonamide and its silver complex

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Sulfonamides with biological activity are known as sulfa drugs (with  $-S(=O)_2-NR_2$  functional group) and treat various bacterial, fungal, and viral infections. The mechanism of effectiveness of sulfonamides is the structural similarity between sulfonamide and  $p$ -aminobenzoic acid (PABA). The present study aims to Compare the Antibacterial activities of N-(2,5-Dihydro-pyrimidine-2-yl)-4-methyl-benzenesulfonamide (L) and its silver complex ( $C_1$ ) by determining MIC.

### MATERIALS AND METHODS

All purchased chemicals were of reagent grade and used without further purification. N-(2,5-Dihydro-pyrimidine-2-yl)-4-methyl-benzenesulfonamide (L) was synthesized from the reaction of 2-Amino Pyrimidine and 4-methyl benzenesulfonyl chloride in dichloromethane solvent one-pot synthesis and under reflux conditions (at a temperature of 60°C) for 8 hours, without a catalyst. The progress of the reaction was followed by thin-layer chromatography (TLC). Then the precipitate was washed with ethyl acetate and distilled water at a ratio of 1:2; methanol/Acetonitrile recrystallized the precipitate. 8 mmol of N-(2,5-Dihydro-pyrimidine-2-yl)-4-methyl-benzenesulfonamide (L) was dissolved in Deuterium-depleted water and ethanol (20 mL, 1:1). 8 mmol of Silver nitrate was added into the solution of ligand and stirred the reaction mixtures for 24 hours at room temperature. The precipitates were filtered and kept at 4°C until the day of use. Antibacterial effects of the synthesized sulfonamide compounds, the minimum inhibitory concentration of bacterial growth (MIC) was determined based on the CLSI reference protocol and by microdilution method in a 96-well microplate using Mueller Hinton Broth. The microbial strains required in this study were purchased from the Iranian Biological Resource Center, including *Escherichia coli* (IBRC-M 10871), *Pseudomonas aeruginosa* (IBRC- M 10205), *Staphylococcus aureus* (IBRC- M 10690), *Bacillus subtilis* (IBRC- M 10742).

### RESULTS AND DISCUSSION

N-(2,5-Dihydro-pyrimidine-2-yl)-4-methyl-benzenesulfonamide and its silver complex were characterized with FT-IR, <sup>1</sup>HNMR, and Single Crystal Crystallography. The order of activities against *E.coli*, *P.aeruginosa*, *S.aureus*, and *B.subtilis* with MIC of L was 1.25, 0.312, 1.25, and 10 mg/ml, and for  $C_1$ , 0.078, 0.078, 0.078, and 2.5 mg/ml, respectively.

### CONCLUSION

It was shown that L and  $C_1$  have moderate to significant activity against all bacteria types and have more inhibitory effects on Gram-negative than Gram-positive bacteria.

**Keywords:** Sulfonamide, N-(2,5-Dihydro-pyrimidine-2-yl)-4-methyl-benzenesulfonamide, Antimicrobial Evaluation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Biocontrol potency of *Bdellovibrio bacteriovorus* toward exo-biopolymer producing phytopathogens: in vitro and in vivo assessments

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Bdellovibrios are predatory bacteria that invade other live Gram-negative bacterial cells for growth and reproduction. They have recently been considered as potential living antibiotics and biocontrol agents. In this study, the predatory activity and biocontrol potency of *Bdellovibrio bacteriovorus* strain SOIR-1 against *Pantoea* sp. strain BCCS and *Xanthomonas campestris*, two exobiopolymer-producing phytopathogens, was evaluated.

### MATERIALS AND METHODS

Plaque formation assays and lysis analysis in the broth co-cultures were used for the in vitro evaluation of bacteriolytic activity of strain SOIR-1. The in vivo biocontrol potential of strain SOIR-1 was evaluated by pathogenicity tests on the onion bulbs and potato tuber slices. The phytopathogens were also recovered from the infected plant tissues and confirmed using biochemical tests and PCR-based 16S rRNA gene sequence analysis.

### RESULTS AND DISCUSSION

Typical bdellovibrios plaques were developed on the lawn cultures of *Pantoea* sp. BCCS and *X. campestris*. The killing rate of strain SOIR-1 toward *Pantoea* sp. BCCS and *X. campestris* was 84.3% and 76.3%, respectively. Exo-biopolymers attenuated the predation efficiency of strain SOIR-1 up to 10.2–18.2% (*Pantoea* sp. BCCS) and 12.2–17.3% (*X. campestris*). The strain SOIR-1 significantly reduced rotting symptoms in the onion bulbs caused by *Pantoea* sp. BCCS (69.0%) and potato tuber slices caused by *X. campestris* (73.1%).

### CONCLUSION

Although more field assessments are necessary, strain SOIR-1 has the preliminary potential as a biocontrol agent against phytopathogenic *Pantoea* sp. BCCS and *X. campestris*, especially in postharvest storage. Due to the particular physicochemical properties of evaluated exobiopolymers, they can be used in the designing encapsulation systems for delivery of bdellovibrios.

**Keywords:** *Bdellovibrio*. Lytic activity. Phytopathogens. Exo-biopolymer. Biological control. Pathogenicity tests

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Design, Synthesis, and Antimicrobial Evaluation of 1-tosyl-1H,1,2,4-triazol-5-amine

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Today, diseases caused by bacteria and fungi adversely affect society's health. Antibiotics are the most common drugs used worldwide and an essential part of treatment, and they are considered critical drugs in the treatment and prevention of Infectious Diseases. The present study aims to synthesize new N-heteroaryl sulfonamide compounds to control and eliminate bacterial and fungal species.

### MATERIALS AND METHODS

All purchased chemicals were of reagent grade and used without further purification. 1-tosyl-1H,1,2,4-triazol-5-amine (L) was synthesized from the reaction of 3-amino-1,2,4-triazole and 4-methyl benzene sulfonyl chloride in dichloromethane solvent one-pot synthesis and under reflux conditions (at a temperature of 60°C) for 8 hours, without a catalyst. Then the precipitate was washed with ethyl acetate and distilled water at a ratio of 1:2; methanol/Acetonitrile recrystallized the precipitate. FT-IR, <sup>1</sup>H-NMR, characterized the prepared L. Single X-ray crystal structure analysis determined molecular and crystal structures. The antimicrobial activity includes the evaluation of the antibacterial effects of the L, including the evaluation of the antibacterial activity of Disk Diffusion (by the Kirby-Bauer method) by measuring the diameter of the growth inhibition zone. The microbial strains required from the Iranian Biological Resource Center Was purchased. The bacterial suspension was prepared equaled 0.5 McFarland in the disk diffusion method. Incubation was done at 37°C for 24 hours. This study used the disk containing gentamicin (10µg) as a positive control, and the disk containing dimethyl sulfoxide as a negative control.

### RESULTS AND DISCUSSION

1-tosyl-1H,1,2,4-triazol-5-amine (C<sub>18</sub>H<sub>20</sub>N<sub>8</sub>S<sub>2</sub>O<sub>4</sub>), IR (KBr) (ν, cm<sup>-1</sup>): 3400 (NH<sub>2</sub>), 3110 (C-H aromatic), 1628 (-C=N), 1504 (C=C aromatic), 1368-1207 (-N-S(=O)<sub>2</sub>). The antibacterial activity against *E.coli*, *P.aeruginosa*, *S.aureus*, and *B.subtilis* with the diameter of the growth inhibition zone of L was 15.9±0.2, 17±0.24, 18.4±0.17, and 10±0.22, respectively. The results were compared with those of Gentamycin. It was observed that the L showed moderate to significant activity against all Gram-negative and Gram-positive bacteria.

### CONCLUSION

Based on the data obtained in this test, L showed more inhibitory effect on Gram-negative bacteria (*E.Coli*, *P.aeruginosa*) than Gram-positive bacteria (*S.aureus*, *B.Subtilis*).

**Keywords:** Sulfonamide, 1-tosyl-1H,1,2,4-triazol-5-amine, Antimicrobial Evaluation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Serological Responses of *Brucella Melitensis* Vaccine Rev-1 Strain in Lambs

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Brucellosis is a common infectious disease between humans and livestock and is still a major problem in most parts of the world. *Brucella melitensis* Rev-1 vaccine is one of the effective vaccines for preventing and controlling brucellosis in young lambs. In this study, serological responses of Razi institute's *Brucella* Rev-1 vaccine and its comparison with the commercial Spanish vaccine (CZV) were evaluated.

#### MATERIALS AND METHODS

12 female sheep (5-8 months old) free of brucellosis were divided into 3 groups of 4. One group was injected as a control by injection of physiological serum and the other two groups with *Brucella melitensis* vaccine made by Razi Institute and Spanish *Brucella melitensis* (CZV) vaccine, respectively. Blood samples and sera were collected from all groups in 0, 2, 4 and 6 weeks after vaccination. All animals with brucellosis strain 16M were challenged after six weeks of vaccination. Then, all animal serum samples were evaluated by Rose Bengal (RBPT), Wright (SAT) and 2ME (2-Mercaptoethanol) and ELISA assay.

#### RESULTS AND DISCUSSION

The results showed that the animals in the control group were serologically negative in all experiments during the 6 weeks before the challenge experiment. The first positive serologic reaction based on RBPT, SAT and 2ME test results was recorded in the second week in both groups and was positive up to 6 weeks after vaccination. In the fourth week, agglutination titer increased in both groups vaccinated with domestic or commercial CZV vaccine and there was no significant difference between both groups with serologic results of SAT and 2ME ( $P > 0.05$ ). The results of the challenge experiment showed that all vaccinated and control groups showed positive serological responses at weeks 2, 4 and 6 with increased agglutination titer.

#### CONCLUSION

The positive serological reactions of the vaccine group (made by Razi Institute) were similar to the positive reactions in the commercial vaccine (CZV) group. It seems that due to similar results of two vaccines in stimulating the humoral immune system of young lambs affect the function of CD4 Th2 type lymphocytes and are currently under investigation.

**Keywords:** Vaccine, *Brucella melitensis*, Serology.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of The Antifungal Effect of Poisons Used in Pistachio Orchards in Qazvin

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

One of the most valuable agricultural assets are pistachio trees which are contaminated by various types of fungal agents. Fungi cause drying of green leaves during the growing season, gum secretion from the crown of the tree, and root rot. To eliminate fungal contamination, fungicides are used abundantly without considering their effects. In Iran, three types of anti-fungal compounds, Previcur, Elite, and Bordeaux, are used to eliminate fungal contaminations. This study aims to investigate the antifungal activity of three types of poisons on isolated fungi from the leaves and roots of pistachio trees in Qazvin.

### MATERIALS AND METHODS

The parts of the root and leaves of the trees suspected of having fungal contamination were separated from the tree and transferred to the PDA medium. After the growth of the fungi in the culture medium, colonies form and microscopic characteristics were evaluated. The efficiency of each fungicide was evaluated with a method of mixing with the culture medium. The equal pieces of the PDA, containing the isolated fungi, were transferred to the PDA containing different concentrations of three types of fungicide, individually. The plates were incubated for a week at 30°C. The growth of the fungi was evaluated and was reported.

### RESULTS AND DISCUSSION

Based on microscopic observations and colony structure, two types of fungi, with characteristics of *Aspergillus* Sp. were identified. The highest antifungal effect was observed when using the Previcur toxin by 3% in the isolated fungi from the leaves of pistachio trees. None of the three poisons had an antifungal effect in reducing the growth rate of the fungus colony isolated from the tree root. Results approved many of the poisons that used in the pistachio orchards have no efficiency to eliminate microbial contaminants. Najarpour et al., have also reported employing salt compounds in different types and concentrations to control the contaminations in pistachio trees should be evaluated to avoid environmental pollution.

### CONCLUSION

Indiscriminate use of pesticides to eliminate fungal contamination without considering the effectiveness of pesticides will not eliminate contaminations but will cause irreparable damage to pistachio trees and the environment. Therefore, it seems necessary to reflect on the efficiency of pesticides before use.

**Keywords:** Antifungal effects, Poisons, Pistachio trees, *Aspergillus*, Environment

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Using minimum inhibitory concentration to evaluate the effect of *Bacillus subtilis natto* as a probiotic on enteropathogenic bacteria

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

Probiotics play a critical role to treat the diarrhea caused by bacteria is a progressing phenomenon. in which probiotics play an essential role. The aim of this study is to investigate the antibacterial effect of the probiotic *Bacillus subtilis natto* on a number of common pathogens of the digestive system.

### MATERIALS AND METHODS

In this study, *Bacillus subtilis natto* strain ATCC G<sub>8</sub>S<sub>3</sub> was selected as probiotic. its antimicrobial effect on four gastrointestinal pathogens, including *Bacillus cereus*, *Salmonella typhimurium* ATCC 1429, *Shigella flexneri* ATCC 11222 and *Escherichia coli* ATCC 25922 was investigated. The minimum inhibitory concentration or MIC method was used to detect the antibacterial activity of *Bacillus subtilis natto* as a probiotic. In this method, 5 different supernatant concentrations including 100, 50, 25, 12.5 and 6.25 (µg/ml) were used. In the next step, minimum bactericidal concentration were performed according to microbiology standards with four repetitions.

### RESULTS AND DISCUSSION

MIC concentration for *Escherichia coli* ATCC 25922 was 50, while no antibacterial effect was observed on *Bacillus cereus* ATCC 11778, *Salmonella typhimurium* ATCC 1429 and *Shigella flexneri* ATCC 11222. Therefore, the results showed that *Bacillus subtilis natto* ATCC G<sub>8</sub>S<sub>3</sub> has an inhibitory effect on some pathogens, including *Escherichia coli*.

### CONCLUSION

Because *Bacillus subtilis natto* ATCC G<sub>8</sub>S<sub>3</sub> is safe and resistant as a probiotic and it was able to show inhibitory results on the pathogen *Escherichia coli*, it can be used for some bacteria in the future

**Keywords:** Probiotics, *Bacillus subtilis*, MIC, Gastrointestinal pathogens

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluation of resistance pattern of microbes isolated from patients with fever and neutropenia admitted to the oncology ward of Bushehr Persian Gulf Martyrs Hospital in Bushehr from 1400 to Shahrivar 1401

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

Fever and neutropenia will have serious effects on the treatment system and the patient by prolonging the hospitalization process, forcing the dose reduction of chemotherapy drugs, taking multiple antibiotics and finally increasing the costs.

#### MATERIALS AND METHODS

This descriptive-analytical cross-sectional study was conducted on 21 patients with fever and neutropenia hospitalized in the oncology department of the Persian Gulf Martyrs Hospital in Bushehr from April 1400 to September 1401.

#### RESULTS AND DISCUSSION

In the examination of the cultures sent from the patients, gram-negative bacteria prevailed and simultaneously, several types were resistant to antibiotics. High antibiotic resistance was observed in the family of cephalosporins and fluoroquinolones. On the other hand, aminoglycosides were the most sensitive. According to the investigation, in 76% of our patients, the site of involvement was unknown, and Gram-negative organisms were predominant in the positive samples.

#### CONCLUSION

According to the pattern of antibiotic resistance, it is recommended to send suitable samples for culture from all suspicious areas when starting antibiotics, and to pay attention to the microbial pattern in order to reduce costs and indiscriminate use of different antibiotics.

**Keywords:** cancer, neutropenia, antibiotics

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Ca<sub>19</sub>Zn<sub>2</sub>(PO<sub>4</sub>)<sub>14</sub> Nanoparticles: Synthesis, characterization and its effect on the colonization of *Streptococcus mutans* on tooth surface

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

The main cause of tooth decay is the biofilm formation of *Streptococcus mutans*. This study aimed to investigate the effect of the zinc oxide nanoparticles (ZnO NPs), hydroxyapatite nanoparticles (Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>) (HAP NPs), zinc oxide/hydroxyapatite nanocomposites (ZnO/HAP NCs), and Zn substituted hydroxyapatite nanoparticles (Ca<sub>19</sub>Zn<sub>2</sub>(PO<sub>4</sub>)<sub>14</sub> NPs) on the growth and biofilm formation, bacterial adherence, and the expression of *ftf* and *gtfC* genes in *Streptococcus mutans*.

### MATERIALS AND METHODS

The nanostructures were prepared via simple and fast co-precipitation route. Twelve isolates of *Streptococcus mutans* collected from children with dental caries referred to the dental clinic of Kashan University of Medical Sciences. All *S. mutans* isolates were susceptible to Ampicillin.

### RESULTS AND DISCUSSION

The mean MIC for ZnO NPs, HAP NPs, Ca<sub>19</sub>Zn<sub>2</sub>(PO<sub>4</sub>)<sub>14</sub>, and ZnO/HAP NCs were 118, 260, 70.6, and 994 mg/mL, respectively. All prepared nanostructures significantly reduced biofilm formation at MIC and sub-MIC concentrations ( $p < 0.01$ ). In biofilm and cell culture treated with nanoparticles, the expression of *ftf* and *gtfC* genes decreased. Results were shown that IC<sub>50</sub> for the Ca<sub>19</sub>Zn<sub>2</sub>(PO<sub>4</sub>)<sub>14</sub> was 8.5, and for non-toxic concentration, was 0.065 mg/mL. The attachment rate to the denture surface and HGF1 cell line treated with the Ca<sub>19</sub>Zn<sub>2</sub>(PO<sub>4</sub>)<sub>14</sub> NPs has decreased.

### CONCLUSION

The results showed that the Ca<sub>19</sub>Zn<sub>2</sub>(PO<sub>4</sub>)<sub>14</sub> NPs has a better effect than the ZnO/HAP NCs. It can therefore be used as a coating on dental surfaces. Investigation of Ca<sub>19</sub>Zn<sub>2</sub>(PO<sub>4</sub>)<sub>14</sub> NPs form for covering dental teeth surface recommended in future study.

**Keywords:** Hydroxyapatite ,Nanocomposites ,Biofilm ,MIC ,Dental

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antioxidant, anticancer, and antibacterial effects of epsilon-poly-L-lysine produced by *Paenibacillus polymyxa* HS6 as a novel strain

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Epsilon-Poly-L-lysine ( $\epsilon$ -PL) is a natural cationic homopolypeptide consisting of at least ten lysine residues linked together by peptide bonds between the  $\alpha$ -carboxyl and the  $\epsilon$ -amino groups. The  $\epsilon$ -PL is biodegradable, water-soluble, edible, and non-toxic to humans and the environment with heat stability and unique structure characterization which have made it a crucial biopolymer in medicine and industries. This cationic biopolymer and its derivatives offer a wide range of applications such as food preservatives, dietary supplements, biodegradable fibers, drug carriers, gene carriers, and anticancer enhancer agents. Since the first report of  $\epsilon$ -PL production by *Streptomyces albulus* 346 as an extracellular product, several  $\epsilon$ -PL producer microorganisms such as *Streptomyces noursei*, *Streptomyces diastatochromogenes*, *Kitasatospora kifunense*, and *Bacillus subtilis* have been introduced so far, but the identification of novel strains with higher production rate is still ongoing. In addition, due to its efficient functional groups and positive charge,  $\epsilon$ -PL can be a potent microbial candidate as an antimicrobial, anti-oxidant, and anticancer agent.

### MATERIALS AND METHODS

In this study, we isolated and identified *Paenibacillus polymyxa* HS6 (accession number: MW791431) from soil samples as a novel  $\epsilon$ -PL producer with a maximum yield of 1.801 g.L<sup>-1</sup>. Furthermore, the antibacterial activity, antioxidative capacity, and cytotoxicity effects of  $\epsilon$ -PL produced by isolate were examined. To obtain pure cationic polypeptide, chemical precipitation with sodium tetraphenylborate (NaTPB) was used. The purified cationic compound was confirmed to be  $\epsilon$ -PL by High-Performance Liquid Chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), <sup>13</sup>C nuclear magnetic resonance (<sup>13</sup>C NMR), and Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE). The antioxidant activity of the purified  $\epsilon$ -PL was then determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Moreover, the anticancer and cytotoxic effects were evaluated against MCF-7, HT-29, and L929 cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and flow cytometry. In addition, the antibacterial activity of  $\epsilon$ -PL was evaluated against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus*

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*faecalis* ATCC 29212, *Serratia marcescens* ATCC 13880, and *Klebsiella pneumoniae* ATCC 13883 by microdilution method.

### RESULTS AND DISCUSSION

The maximum yields of 1.8 g/l  $\epsilon$ -PL were obtained by *P. polymyxa* HS6 after 20 hours of incubation in M3G culture medium. The results showed that the antioxidant capacity of  $\epsilon$ -PL was concentration-dependent, and there is a significant correlation between concentration and radical scavenging activity. Radical scavenging activity of  $\epsilon$ -PL at a minimum concentration (0.18 mg/mL) was at least 7% which increased to 90% at 8 mg/mL. Furthermore, the highest anticancer activity was observed against the MCF-7 cell line (99.5%) at 0.5 mg/mL concentration, while almost no toxicity was recorded towards L929 cells. Also, the results showed that  $\epsilon$ -PL in less than 2 mg/ml has a great antibacterial effect and can inhibit the growth of the bacteria.

### CONCLUSION

In spite of tremendous medical and pharmaceutical research progress, combat with cancers and infections is still one of the most global public health challenges. Some of the natural compounds with hopeful bioactive properties represent possible candidates to overcome the disadvantages and side effects of chemical and synthetic agents. Based on the results obtained in this study, it is suggested that the  $\epsilon$ -PL produced by *P. polymyxa* HS6 is a potential bioactive compound with significant anticancer, antioxidant, and antibacterial properties.

**Keywords:** Bioactive compound, Microbial polymers, Cationic polypeptides, epsilon-poly-L-lysine

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# Combination of built-in “TLR4/5 agonists/tetanus toxoid and TLR7 agonist/alum adjuvants elicits robust Th1/Th2 anti-HPV L2 RG-1 responses in immunized mice

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Human papillomavirus (HPV), a heterogeneous group of around 200 types that infect the epithelia of the skin and mucosa in humans, is the most common sexual infection and the main cause of cervical cancer. Currently available HPV vaccines (Cervarix and Gardasil/9) are highly efficient in the prevention of infection due to induction of type-specific and high titer neutralizing antibody (nAb) against major capsid protein (L1) of the virus. Although these vaccines protect from major cancer producing HPV types such as 16, 18, 31 and 45, but type-specificity (limited cross-protections), high cost and technical complexity of the production process, restrict their versatile utilization especially in developing countries. Several prior studies indicated that HPV minor capsid protein (L2) contains a conserved pan-type linear so called RG1 epitope (amino acids 17-36) capable of eliciting broadly cross-reactive nAbs against different HPV types, albeit in much lower titers and potency compared to the L1-based VLP. To this end, several strategies such as multiplication of the RG1 epitope and using various adjuvants were employed to enhance its immunogenicity.

### MATERIALS AND METHODS

Herein, we designed a construct harboring the dual 3x tandem repeats of the HPV16 RG1 epitope (TP), the Entolimod (the TLR5 agonist), the short peptide “RS09 (the TLR4 agonist)” and the tetanus toxoid P2 epitope (TT-P2) which were linked by the (GGGS)<sub>3</sub> linker in tandem, and evaluated for immunogenicity in BALB/c mice with different adjuvant formulations.

### RESULTS AND DISCUSSION

Analyses by SDS-PAGE and Western blot indicated that the expression of this construct in *E.coli* produced the expected 46 kDa protein (hereafter; rejoined peptide (RP)). Groups of mice were immunized (either subcutaneously (SC) or intramuscularly (IM) by RP alone and TP or RP formulated with different adjuvant formulations containing TLR7 agonists (in the form of either injectable R837 imiquimod or Aldara imiquimod cream (AL) for skin treatment) and Alum adjuvants. Assessment of the humoral responses and IFN- $\gamma$  secretion as well as cellular proliferation showed that mice immunized by RP-alone or RP-containing formulations (RP+Alum+R837, RP+Alum, RP+R837, RP) induced significantly higher Nabs and higher IFN- $\gamma$  secretion and cellular proliferation compared to those immunized by any TP-containing formulations (TP+Alum+R837, TP+Alum, TP+R837, TP).

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These results indicated the pivotal role of the designed built-in adjuvant within the RP immunogen for induction of anti-RG1 responses. Moreover, the highest level of humoral and cellular responses (IFN- $\gamma$  secretion and cellular proliferation) was reached in mice immunized SC by “RP+TLR7 agonist/alum adjuvants” formulations (RP+Alum+R837 and RP+Alum+AL) compared to other RP-immunized mice groups. This observation indicated that TLR7 agonists (R837 or Aldara) /alum adjuvants synergistically enhanced the crucial effect of built-in adjuvants on the induction of anti-HPV RG1 immune responses. Moreover, assessment of IgG1/IgG2a ratios indicated that groups receiving TLR7 agonists (R837 or Aldara) by SC immunization (RP+Alum+R837, RP+Alum+AL or RP+R837) showed a balanced Th1/Th2 polarization of the immune system, while groups deprived of TLR7 agonists or received TLR7 (R837) via IM immunization route showed a Th2 phenotype.

### CONCLUSION

Collectively, our data showed the synergistic effect of TLR7 agonist/alum to enhance the crucial role of the built-in “TLR4/5 agonists / (TT)-P2” for induction of robust and balanced Th1/Th2 anti-HPV RG1 immune responses. Results of this study might help to achieve a promising vaccine formulation for the induction of anti-HPV L2 RG1 immune responses for production of an inexpensive pan-genomic and cross-reactive HPV vaccine.

**Keywords:** HPV, RG1 epitope, Built-in adjuvants, Tetanus toxoid, Neutralizing antibody.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The Effect of Microencapsulated Native Lactobacillus on Gut Gram-negative Bacteria Flora of Diabetic Male Rats

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Diabetes is a chronic and complex metabolic disorder that is regarded as one of the leading causes of death in the world. This major problem affects the health of millions of people in all age groups in both genders around the world, the outbreak of which reached 49% in 2010 in the age group of 20 to 79. Consequently, it is expected that it will reach 77% by 2030. According to the World Health Organization, around 171 million people worldwide develop it, and it is expected this number will rise by 366 million by 2030. Therefore, the search for new therapeutic resources and prevention methods is high on the agenda for the researchers. As the use of medications can have serious side effects, it would be beneficial to use natural compounds in foods for treatment. Probiotics have been selected as live microorganisms, which are potentially useful as food supplements for both humans and animals, and are used in human health improvement and disease prevention.

### MATERIALS AND METHODS

In this study, samples were taken from the intestine of four groups of diabetic male rats and of the control groups. Then, probiotic bacteria and Escherichia coli bacteria were counted in MRS and EMB agar medium

### RESULTS AND DISCUSSION

The results showed that oral administration of lactobacilli in diabetic group was significantly different from that of the diabetic control group in the number of lactobacillus. The number of Escherichia coli was also significantly lower in the treated diabetic group than that in the diabetic control group

**Keywords:** Diabetes, Intestinal microbial flora, Probiotics, Lactobacillus, Escherichia coli

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# The Study of antibacterial effects of five Iranian plant Methanolic extracts on *Helicobacter pylori* isolates Resistance in East Azerbaijan province

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### ABSTRACT

#### BACKGROUND AND OBJECTIVE

*Helicobacter pylorus* is considered as an important human pathogen which is associated with a variety of gastrointestinal diseases, such as chronic gastritis, peptic ulcers, stomach cancer and MALT lymphoma. So, *H. pylori* eradication is recommended being the most effective method for healing gastric ulcers. In fact, the main reason for failure was recognized to be *H. pylori* resistance to antibiotics. Accordingly, the purpose of this research was to study the antibacterial effects of five Iranian plants on *H. pylori* isolates resistance to metronidazole.

#### MATERIALS AND METHODS

A biopsy specimen was obtained from each of 100 or 268 patients with dyspepsia referred to hospital. Then, they were transferred to the laboratory in transport medium (1% agar). The biopsies were first defibrinated in Brucella agar medium containing 7% sheep blood, and vancomycin (5mg/L), polymyxin B (50mg/μg) and amphotericin B (4mg/L) were cultured. Then, the plates were incubated at 37°C under microaerobic conditions (CO<sub>2</sub> incubator). Urease, catalase and oxidase tests were used for identifying *H. pylori*. A bacterial suspension matching the turbidity of a 2 McFarland standard was poured on the plates. When the surface of the medium was dried, sterile paper discs (already autoclaved) were placed on the middle of the medium with the help of forceps. 10 microliter of the antibiotic dilution was slowly inoculated on paper disc with Sampler. The zone of bacteria inhibition was observed after 3 days of incubation in anaerobic condition. Antibacterial effects of five plants' extracts were tested on resistant bacteria to antibiotics metronidazole.

#### RESULTS AND DISCUSSION

Resistance rate to metronidazole was 65%; to ofloxacin, 26%; to ciprofloxacin, 17%; to levofloxacin, 15%; to clarithromycin, 19%; to amoxicillin, 13%; to tetracycline, 12%; and to furazolidone, 5%. Among *Chamaemelum*, *Glycyrrhizaglabra*, *Rosmarinus*, *Hypericum perforatum*, *Achillea millefolium*, *Rosmarinus* had the most impact on the antibiotic-resistant bacteria.

#### CONCLUSION

According to the results of the study and increasing resistance to synthetic antibacterial drug, *Rosmarinus* plant may be one of the most effective one in removing some bacteria including *H. pylori*.

**Keywords:** *Helicobacter pylori*, Drug Resistance, Iranian Plant, Antibacterial

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The effect of starch on biosurfactant production in *Lactobacillus casei*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Biosurfactants are amphiphilic compounds with many applications as detergents, emulsifiers, and foaming agents. In recent years, microbial biosurfactants have received a lot of attention compared to chemical surfactants due to their diversity and unique characteristics, including biodegradability, and low toxicity, and have replaced chemical surfactants such as sodium lauryl sulfate in detergents. *Lactobacillus* species are capable of producing biosurfactants, which have great potential for use in various industries, or as food ingredients. Low production efficiency is one of the problems to run large scale fermentations.

#### MATERIALS AND METHODS

In this study, the effect of starch to increase biosurfactant production in *Lactobacillus casei* was investigated. Biosurfactant production by *Lactobacillus casei* in 200 cc of MRS broth and 1% starch was investigated after 48, 72 and 96 hours at 37 ° C and compared with the starch-free medium. In order to extract the biosurfactant from cell-free extract, bacterial cells were first removed from the broth by centrifugation at 3800 rpm for 25 min. Then the pH of the cell-free broth was set at 2.0 with concentrated HCl and were saved at 4°C for 30 min. The samples were centrifuged for 25 min at 3800 rpm and the broth was discarded. The pellets were washed and centrifuged with PSB. After that, the pellets were resuspended with 2.0 mL of distilled water. The dry weight of sediment was obtained and compared in both mediums.

#### RESULTS AND DISCUSSION

The results showed that 1% starch could increase the amount of biosurfactant produced by *Lactobacillus casei*. According to the results, 0.14, 0.30, and 0.38 mgr biosurfactants were obtained from MRS medium after 48, 72 and 96 hours of incubation. The highest amount of biosurfactant production in this bacterium was obtained in starch containing MRS medium after 96 hours (0.38 mgr), which showed a 10% -fold increase.

#### CONCLUSION

Usage of starchy substances as cheap renewable additives in the fermentation medium of *Lactobacillus casei* can increase the biosurfactant production and reduce costs on an industrial scale.

**Keywords:** Biosurfactant, starch, Probiotics, *Lactobacillus casei*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigation of airborne bacterial abundance and their diversities during occurring fine dust storms in Lorestan province

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Dust storms are natural phenomena occurring more in arid and semi-arid regions that have adverse effects on ecosystems and human health. Dispersion of microorganisms via dust storms to a faraway is one of the important aspects of microbial ecology and air microbiology. The aim of this study was to investigate the airborne bacteria abundance and its diversity during occurring dust storms in some cities of Lorestan province using the classical methods.

#### MATERIALS AND METHODS

In the cross-sectional descriptive study, sampling of air dust were carried out in three cities of Lorestan province with different geographical locations. The sampling was performed according to the standards of the American Environmental Protection Agency (EPA) by passive sedimentation method on R2A, TSA culture media. Airborne bacteria were isolated and identified using classical methods and the data analysis was performed by Chi-square test. The relationship between bacterial abundance and meteorological parameters were investigated and analyzed using Spearman's correlation test.

#### RESULTS AND DISCUSSION

In this study showed that 200 colonies grew on TSA medium. Of these colonies 67.5% were bacteria and the rest (32.5%) were yeast. The microbial frequency at Khoramabad station was the lowest at 3% and at kohdasht station was the highest (31.5%). On R2A culture medium, 196 colonies grew that 67.8% of these colonies were bacteria and 32.2% were yeast. Poldakhtar station had the highest microbial frequency with 26.5% and Khorramabad station had the lowest bacterial frequency at 9.2%. In TSA and R2A culture media, the highest isolates were *Bacillus* and the lowest one was *Staphylococcus*. Furthermore, the highest microbial frequency was detected in Kohdasht and Poldakhter. Based on the Spearman correlation test, a direct relationship was observed between the amount of dust and the abundance of bacteria ( $r = 0.581$ ,  $p_v = 0.045$ ), while an inverse linear relationship was observed between the wind speed and the number of colonies ( $r = -0.619$ ,  $p_v = 0.038$ ). There was no significant relationship among temperature, humidity and abundance of bacteria. Moreover, identification of yeast strains were not the object of this study.

#### CONCLUSION

Bacterial abundance was higher in R2A culture medium. Gram-positive bacteria were isolated in both TSA and R2A media. In this study no gram-negative bacteria were isolated. *Bacillus* and *Micrococcus* were the dominant bacteria in both R2A and TSA culture media. One of the main reasons for presence of these bacteria is formation of spore and production of pigments. Both these characteristics help these microorganisms to survive in dryness and other harsh environmental conditions. It can be concluded that the amount of fine dust and wind speed affected the abundance and diversity of airborne bacteria.

**Keyword: Fine Dust, Lorestan, Meteorological Parameters, Microbial Contamination**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating the antimicrobial and antibiofilm effects of clove extract on clinical isolates of *Pseudomonas aeruginosa* in Kashan

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

*Pseudomonas aeruginosa* bacteria is one of the main causes of nosocomial infections, which is considered as important public health challenges. *Pseudomonas aeruginosa* causes infections in different parts of the body such as the respiratory tract, urinary tract, and digestive tract. These infections include bacteremia, septicemia, pneumonia, skin infections and wounds, etc. The spread of drug-resistant *Pseudomonas aeruginosa*, especially to multi drugs resistant (MDR), has led to an increase in complications and death caused by infections of this pathogen. One of the main reasons for resistant bacteria is the formation of biofilm, which increases their resistance to most classes until its resistance is 100 times the initial resistance.

#### MATERIALS AND METHODS

In this cross-sectional-descriptive study, all of 40 isolates of *Pseudomonas aeruginosa* was isolated from different clinical specimen (blood, wound, cerebrospinal fluid (CSF), urine, sputum, bronchoalveolar lavage, synovial fluid, pleural fluid, etc.). The bacteria isolates to surgery, intensive care unit (ICU), critical care unit (CCU), pediatrics, internal medicine, emergency, obstetrics and gynecology, otolaryngology and outpatient departments of Shahid Beheshti Hospital in Kashan were collected. Primary identification based on oxidase, catalase, triple sugar iron, fermentation of various sugars, citrate test, indole, VP, MR, growth at 42 degrees Celsius, color, production of pigments, investigation of movement on SIM environment and specific smell were done. Then Polymerase chain reaction (PCR) as molecular method using *oprL* gene was used for final confirmation. Then, isolates of *Pseudomonas aeruginosa* producing biofilm were identified by microtiter plate assay. Finally, the antibacterial and anti-biofilm effect of clove extract was investigated in these bacteria.

#### RESULTS AND DISCUSSION

The size of the band observed in PCR was 193 base pair, which was the molecular confirmation of *Pseudomonas aeruginosa* bacteria. In this study, 23 isolates (57.5) that have the ability to form a strong biofilm, 10 moderate isolates (26%) and 7 weak isolates (16.5%). The most bacteria isolated able to produce strong biofilm were from the ICU department (23.8%). The minimum inhibitory concentration (MIC<sub>100</sub>) of clove extract is 62.5-125 mg/ml and the

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minimum biofilm inhibitory concentration (MBIC<sub>100</sub>) was 31.25-62.5 mg/ml. Our results demonstrate that MDR *Pseudomonas aeruginosa* produce more biofilm. Altered changes in quantity of biofilm formation were detected to be affected clinical isolates with sub-MIC concentrations of clove extract. Today, the main strategy to control and eradicate resistant bacteria producing biofilm is to use chemical biocides and disinfectant solutions, which have many unwanted side effects. Also, common uses are limited due to increased drug resistance. The use of new antimicrobial agents such as herbal plants with less side effects can help us. One of these plant extracts is clove extract, which was observed in this study to have inhibited growth of *Pseudomonas aeruginosa* bacteria. In addition, it's prevented the formation of biofilm.

### CONCLUSION

Using natural extracts instead of chemicals can be helpful in controlling drug-resistant bacteria, especially MDR. It is suggested to investigate the antimicrobial activity of clove extract on other antibiotic-resistant bacteria such as *Staphylococcus aureus*, *Enterococcus*, and *Escherichia coli*.

**Keywords:** *Pseudomonas aeruginosa*, biofilm, antimicrobial activity, clove extract.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the effects of *Klebsiella* uterine infections on the reproductive rate of mares of Kurd and DerehShoor in Kerman province

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

*Klebsiella pneumoniae* causes a wide range of infections and the bacterium has the ability to cause infection in almost any organ of the body. The remarkable point is that out of hundreds of antibiotics produced in nature that have been purified, only a limited number are non-toxic and as a result they are used as antibiotics. One of the important characteristics of this bacterium is its low sensitivity to antibiotics. Placental fetal infections cause one-third of horse abortions. Therefore, the aim of this study was to investigate the effects of uterine infections with *Klebsiella* origin on the reproductive rate of mares of Kurdish breed and DerehShoor of Kerman province.

### MATERIALS AND METHODS

The current study is a descriptive-cross-sectional type of research that was collected in 1402 from 40 adult horses with clinical symptoms. In this research, among forty adult mares of the DerehShoor (20 head) and Kurd (20 head) breeds, with an average age of five to fifteen years, with amber and brown color, which had a previous pregnancy (7-8 foals), for example Respiratory secretions and uterine secretions were used. AntibioGram culture regarding the antibiotic sensitivity of the isolated bacteria was performed based on the CLSI standard. Uterine (vaginal) ultrasound was performed using a 5.7 MHz probe and through the mentioned methods: through the abdomen - through the vagina.

### RESULTS AND DISCUSSION

For the sensitivity of bacteria isolated from all samples, Mueller Hinton Agar (Merck Company, Germany) was used to check the minimum concentration (dilution) of the inhibitor. The investigated antibiotics included amikacin (AM), Norfloxacin (Nor), the most sensitive, cefixime (CFM), Nalidixic acid (NA), sulfamethoxazole (SXT), the most resistant, and nitrofurantoin (FM300).

### CONCLUSION

According to the results of this study, the diagnosis of endometritis after birth and spherogenesis in the clinical environment usually includes examination of the reproductive system within 48 hours after delivery. All mares experience some degree of physiological endometritis, which includes a mechanical or immune response used by the mare to shed excess. Complete treatment with antibiotics is no longer an appropriate strategy and must be evidence-based. The growing interest in non-antibiotic alternatives warrants further investigation, but more data are needed to assess their efficacy and safety.

**Keywords:** Bacterial contamination, *Klebsiella pneumoniae*, mares, endometritis, AntibioGram

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Exploring the Influence of Various Treatments on the Autofluorescence Properties of *Bacillus subtilis*

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Bacterial autofluorescence, characterized by the spontaneous emission of light without external fluorescent labels, offers a unique and non-invasive approach to investigate bacterial metabolic activities and physiological responses. In this study, we investigated the impact of different treatments on the autofluorescence of *Bacillus subtilis*, a versatile model organism widely utilized in biotechnology and research.

### MATERIALS AND METHODS

The effects of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), heat, cold, ethanol, glucose deprivation, and acidic and alkaline conditions on the autofluorescence properties were evaluated. Treated samples were subjected to a 10-minute exposure to 365 nm ultraviolet (UV) light using a fluorescence microscope. Subsequently, the samples were evaluated using a Shimadzu RF-spectrofluorimeter with excitation wavelengths of 365 nm and 405 nm and emission wavelengths ranging from 390 nm to 900 nm.

### RESULTS AND DISCUSSION

Our findings demonstrated that acidic and alkaline conditions had minimal impact on the autofluorescence of *B. subtilis*. However, glucose deprivation and exposure to H<sub>2</sub>O<sub>2</sub> led to a significant reduction in autofluorescence levels, up to 60%. Conversely, ethanol exposure and heat treatment resulted in an increase of up to 50%, while cold conditions induced a substantial 100% increase. Furthermore, we analyzed the lysate and supernatant of *B. subtilis* to assess the presence of autofluorescence. Our results confirmed the presence of autofluorescence in both phases, with the lysate exhibiting significantly higher fluorescence levels compared to the supernatant.

### CONCLUSION

These findings contribute to our understanding of the autofluorescence characteristics of *B. subtilis* and offer potential applications in fields such as fluorescence microscopy and spectrofluorimetry.

**Keywords:** Bacterial autofluorescence, *Bacillus subtilis*, treatment effect investigation

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### A report of neck muscles hemorrhage in an Alpine goat following enterotoxemia; a new necropsy finding

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Enterotoxemia, which sometimes called “overeating disease” or “pulpy kidney”, is caused by *Clostridium perfringens* type D, which severely harms the goat industries economically. It's possible to infect both kids and adult goats. Alpha (CPA) and epsilon (ETX) toxins are produced by *C. perfringens* type D, which normally presents in the intestine of ruminants. The majority of the clinical signs and lesions in goats and sheep are caused by ETX, the major virulence factor. The production of toxin and the number of *C. perfringens* can increase by predisposing factors that alter the intestinal environment, such as high levels of carbohydrates, high-protein diets and grasses, and sudden changes in diet. The current study's objective is to report a brand-new necropsy signs (neck muscle hemorrhage- NMH) that was found in an enterotoxemia-dead goat kid.

#### MATERIALS AND METHODS

After exhibiting clinical signs of acute enterotoxemia, a three-month-old Alpine kid died. Convulsions, a 41°C fever, vocalization, and signs of ventricular pain (tucked abdomen and kyphosis) were clinical signs before death. Soon after the death, a postmortem examination was conducted. Small intestine specially ileum contents were aseptically sampled and sent to the laboratory in 1% chloroform under standard conditions (at 4°C). Intestinal specimen was diluted (1:5) with endotoxin-tested distilled water (Sigma-Aldrich Chemie GmbH, Germany) and centrifuged at 2000×g for 20 min at 4 °C. After centrifugation, supernatants were removed and passed through 0.45-µm membrane filters (Millipore, Bedford, MA, USA) and kept at – 70 °C until used. The commercial Bio-X enterotoxemia ELISA kit (Bio-X Diagnostics, Belgium) was used according to the manufacturer's instructions to detect the enterotoxins of *C. perfringens* (alpha, beta and epsilon) and also to confirm the presence of the bacteria itself in the sample obtained.

#### RESULTS AND DISCUSSION

There were no obvious external lesions on the body, according to a postmortem examination. Hemorrhage and hyperemia of the colonic mucosa and small intestine, pulmonary edema, hemothorax, hydropericardium, and hemorrhage in the neck muscles (NMH) were among the Gross necropsy lesions. The results of ELISA revealed that the intestinal contents contained

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alpha and epsilon enterotoxins as well as *C. perfringens* type D bacteria. Enterotoxemia diagnosis is primarily determined by the animal's history, clinical signs, and sudden demise. Gram-positive *C. perfringens* rod isolation from the intestine does not confirmatory the diagnosis. Enterotoxemia can be definitively confirmed by necropsy lesions and the detection of enterotoxins. Researchers have used ELISA in numerous studies because it has the highest sensitivity and specificity for detecting enterotoxemia, particularly epsilon toxin. The clinical signs before death in addition to the necropsy lesions and ELISA results confirm acute enterotoxemia in this Alpine goat.

### CONCLUSION

The hemorrhagic necropsy lesion in the neck muscles (NMH) that developed after an acute enterotoxemia in an Alpine kid was reported in present study. Goats and sheep have different necropsy lesions in the acute form of enterotoxemia. For instance, focal symmetrical encephalomalacia (FSE) has rarely been observed in acute cases of goat enterotoxemia. Additional research will be needed to determine whether NMH qualifies as a unique gross lesion in goats. Goats are considered highly susceptible to enterotoxemia, therefore, it can only be prevented by adopting a proper vaccination program and a balanced diet.

**Keywords:** Enterotoxemia, Alpine, Neck muscles hemorrhage, *Clostridium perfringens*, Necropsy lesions

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# Investigation of the effectiveness of local probiotic strains on pathogens associated with facial acne. *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus* in laboratory conditions

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

Acne vulgarism or simply acne is one of the oldest skin diseases which forms when hair follicles become clogged with dead skin cells and form hard plugs or swelling on the skin. The formed plugs can become infected with other pathogenic bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, etc. that lead to difficulties in treatment of acne and might increase the treatment period. Moreover, antibiotic treatments used have become undesirable owing to the development of antibiotic resistant strains that results in delay in acne treatment. The main objective of the Present study was to evaluate the antibacterial properties of the selected probiotic Lactic Acid Bacteria (LAB) against acne causing bacteria and note their efficacy as a treatment option for face acne

### MATERIALS AND METHODS

The acne sample were taken aseptically from 12 acne lesions from 15-15 years old male and female volunteers. Samples were collected in sterile Brain Heart Infusion Broth and transferred to laboratory for immediate processing. The pathogenic bacteria were isolated from the collected samples using microbiological and molecular methods. The LAB probiotic used in this study were obtained from Razi type culture collections, Iran, while, *Lactococcus lactis* PTCC 1336 was obtained from Persian type culture collection, Iran. The agar well and disk diffusion assay was used to evaluate the antibacterial activity of the cell free supernatant fluids of selected probiotic bacteria (10<sup>8</sup> CFU/ml) against 10<sup>5</sup>-10<sup>6</sup> CFU/ml of *S. aureus*, *S. epidermidis* and *P. acnes* isolated from acne lesions. Minimum inhibitory and bactericidal concentrations were determined by standard microdilution method. The results of the experiment were analyzed with SAS software (SAS/STAT, 1990) and the comparison of means was done by Duncan's multi-range test method

### RESULTS AND DISCUSSION

Three probiotic strains including *Lactocaseibacillus casei* RTCC 1296-2, *Enterococcus faecium* RTCC 2347 and *Lactococcus lactis* PTCC 1336 showed maximum antagonistic effects against the acne associated pathogens. *L. casei* was the most effective strain showing highest antagonistic effects against *Epidermidisae* with MIC and MBC values of 6.25 and 12.50 µg/ml,

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respectively. Higher MIC and MBC values were recorded for other two pathogens (*S. aureus* and *P. acne*) used in study (12.50 and 25 µg/ml), respectively

### CONCLUSION

Acne and related pathogens can be treated with proper selection of local probiotic strains and determination of their appropriate concentration. However, it is recommended to conduct a study with larger number of acnes associated pathogens and screen the antagonistic activity of larger number of probiotic strains

**Keywords:** Propionic bacterium acne, Treatment, probiotic

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# An RNAi Approach Targeting RNA polymerase Gene of Classical Swine Fever

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Classical swine fever virus (CSFV) is a high consequence pathogen. Infection with highly virulent CSFV isolates generally leads to death of infected animals, whereas isolates of moderate to low virulence induce a chronic disease. Classical swine fever (CSF) is a notifiable disease to the World Organization for Animal Health (OIE). In recent years; gene therapy has become a powerful strategy against viruses. Therefore, in the present study, the possibility of using and designing shRNA against CSFV was investigated.

### MATERIALS AND METHODS

Oligonucleotides encoded siRNA molecules were designed against RNA polymerase gene of classical swine fever using the [www.invivogen.com/sirna-wizard](http://www.invivogen.com/sirna-wizard) online website and the most effective molecules were selected using background information. For this purpose, standard search method selected and siRNA motifs with the desired size and thermodynamic properties were designed. Then, in order to design hairpin, the proposed vector and loop sequences submitted, so the most effective shRNAs with desired restriction enzyme sites were designed.

### RESULTS AND DISCUSSION

Three potentially effective shRNA molecules were designed. Their start target positions included with respectively positions of 453, 501, 583 and 624 of classical swine fever RNA polymerase sequence.

### CONCLUSION

The results showed that there are potentially effective shRNA molecules against RNA polymerase gene of classical swine fever that can suppress its proliferation.

**Keywords:** shRNA , classical swine fever, RNA polymerase gene, RNAi.

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# A Review on the New Indication of Nucleoside Reverse Transcriptase Inhibitors (NRTIs) in the Treatment of Coronavirus Disease 2019

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

An enzyme named reverse transcriptase is found in retroviruses, such as the human immunodeficiency virus (HIV), and produces DNA from the RNA template. This enzyme is inhibited by Nucleoside reverse transcriptase inhibitors (NRTIs). Hence, this class of antivirals is utilized to treat HIV and also HBV infections. These drugs inhibit virus replication *via* blocking reverse transcriptase. In this review, we discuss the interaction of this class of anti- HIV drugs with specific functional proteins and enzymes of SARS-CoV-2.

### MATERIALS AND METHODS

A search of the databases, including Web of Science, Embase, PubMed, Scopus, and Google Scholar, from commencement to September 2020 was done. The keywords include "SARS-CoV-2," "COVID-19," "Coronavirus," "Nucleoside reverse transcriptase inhibitors," "Tenofovir," "Emtricitabine," "Zidovudine," "Stavudine," "Didanosine," "Lamivudine," and "Abacavir." Criteria for inclusion were clinical trials or observational studies regarding the effects of NRTIs on SARS-CoV-2. Twenty-three articles were selected, including *in vitro*, *in vivo*, and clinical studies.

### RESULTS AND DISCUSSION

Studies showed that NRTIs could be effective against SARS-CoV-2 through inhibition of RNA-dependent RNA polymerase (RdRp). Emtricitabine, zidovudine, and abacavir bind to target proteins, spike and Angiotensin-converting enzyme (ACE2), with different binding energies. Didanosine is a promising drug in treating COVID-19 by targeting Purine nucleoside phosphorylase (PNP). Didanosine exerts its anti-inflammatory action *via* inhibiting adenosine kinase function. Moreover, blockade of interleukin-2 receptor by didanosine leads to a reduced immunologic response. Tenofovir was found to reduce the amount of inflammatory cytokines such as interleukin (Il)-6, interferon, Il-10, and monocyte chemoattractant protein-1. Zidovudine as a potential therapy has a strong and stable interaction with nucleocapsid protein (N-protein).

### CONCLUSION

RdRp, spike, ACE2, PNP, inflammatory cytokines, and nucleocapsid protein are involved in the pathogenesis of COVs. As discussed, NRTIs have potential mechanisms against SARSCoV-2 by targeting these cytokines and proteins. However, it is important to prove their effectiveness in clinical trials.

**Keywords:** Coronavirus, SARS-CoV-2, COVID-19, RdRp, nucleoside reverse transcriptase inhibitors, zidovudine

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of infection course in mice induced by *L. major* in presence of positively charged liposomes containing CpG ODN

**Hesamoddin Hosseinjani<sup>1</sup>, Mahmoud Reza Jaafari<sup>1,2</sup>, Ali Khamesipour<sup>3</sup>, Azam Abbasi<sup>1</sup>, Zahra Saberi<sup>1</sup>, Ali Badiiee<sup>1\*</sup>**

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

The ability of CpG oligodeoxynucleotides (CpG ODN) to induce both innate and adaptive cellular immune responses makes it a prospective prophylactic and therapeutic vaccine adjuvant for diseases requiring cellular immunity. In the present study, cationic nanoliposomes containing CpG ODN was used according to their simplicity during formulation and stability issues, to explore the possibility to induce a milder lesion and infection after live *Leishmania* parasites inoculation in susceptible BALB/c mice.

### MATERIALS AND METHODS

BALB/c mice were inoculated subcutaneously with *L. major* plus empty Distearoylphosphatidylcholine (DSPC), DSPC (CpG ODN), DSPC (Non CpG ODN), empty 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), DMPC (CpG ODN), DMPC (Non CpG ODN) or HEPES buffer.

### RESULTS AND DISCUSSION

The results showed that group of mice received DMPC (CpG ODN) nanoliposomes developed a significantly smaller lesion and showed minimum number of *L. major* in the spleen and draining lymph nodes. In addition, using DMPC (CpG ODN) liposomes resulted in a Th1 type of immune response with a preponderance of IgG2a isotype which is concurrent with the production of DMPC (CpG) induced IFN- $\gamma$  in the spleen of the mice. In this study, DMPC (CpG ODN) nanoliposomes enhanced the production of IgG2a and particularly IgG2a/ IgG1 ratio, as a hallmark of Th1 type of immune response which is essential for counteracting intracellular pathogens.

### CONCLUSION

In conclusion, the current results suggested that immune modulation using DMPC (CpG ODN) cationic nanoliposomes might be a practical approach to improve the safety of inoculation of live *L. major* and can be utilized instead of lipid-peptide-DNA (LPD) nanoparticles.

**Keywords:** CpG ODN, DMPC (CpG ODN), Nanoliposomes, Immune response, Leishmanization, *L. major*

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# Distribution of Pathogenicity Islands Among Uropathogenic *Escherichia coli* Isolates From Patients With Urinary Tract Infections

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Uropathogenic *Escherichia coli* (UPEC) is one of the most common etiologic agent of urinary tract infection (UTI). The ability of *Escherichia coli* to cause UTI is associated with specific virulence determinants, which are encoded by pathogenicity islands (PAIs). This study aimed to investigate the distribution of PAIs among the UPEC isolates collected from patients with UTIs.

### MATERIALS AND METHODS

In this study, a total of 100 *E. coli* isolates were collected from patients with UTIs using standard microbiological methods. Polymerase chain reaction (PCR) was used for the identification of the main PAIs of UPEC according to insertion sites and virulence markers.

### RESULTS AND DISCUSSION

In total, PAI IV536, PAI III536, PAI I536, PAI, IICFT073, PAI ICFT073, PAI IJJ96, PAI II536, and PAI IJ96 were detected in 23, 22, 17, 17, 13, 11, 11, and 8% of isolates. PAI combinations were identified in 15% of isolates.

### CONCLUSION

The results showed that PAIs of UPEC are not strain-specific and some strains can carry the PAIs associated with the prototype strains of UPEC simultaneously.

**Keywords:** Pathogenicity island, Insertion site, Urinary tract infections, Uropathogenic *Escherichia coli*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating co-infection of COVID -19 and Mycobacterium tuberculosis.

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#### ABSTRACT

##### BACKGROUND AND ABJECTIVE

During the pandemic of the coronavirus disease (COVID-19), many patients around the world, including Iran, were affected by this virus (1, 2). Tuberculosis also causes many deaths every year. There is limited evidence of an interaction between COVID -19 and pulmonary tuberculosis (3, 4). The aim of this study was to determine the prevalence of simultaneous infection of these two pathogens in Kashan city.

##### MATERIALS AND METHODS

From July 2019 to February 2020, 240 respiratory clinical samples from suspected tuberculosis cases were referred to the Kashan University of Medical Sciences. All samples were evaluated for the presence of Mycobacterium tuberculosis via Ziehl-Neelsen and PCR for 16SrRNA (5). In addition, the presence of COVID -19 was detected using the RT-PCR kit (6). Chi-square test was used to check the relationship between variables (significance level = P-value 0.05).

##### RESULTS AND DISCUSSION

Out of 200 examined samples, Mycobacterium tuberculosis and coronavirus 2019 infections were detected in 89 and 180 samples, respectively. Co-infection was detected in 18 samples. In addition, the findings showed that the risk of death from co-infection was greater than that of pulmonary tuberculosis or COVID-19.

##### CONCLUSION

Although the rate of co-infection of pulmonary tuberculosis and COVID -19 was not statistically significant, the results showed that the mortality rate in patients with co-infection is almost 2 times higher than the mortality rate in people who only had tuberculosis (7). Therefore, the facilitating effect of COVID -19 on the activation of tuberculosis requires further research.

**Keywords:** COVID-19, Tuberculosis, interaction, pulmonary tract, Kashan.

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# A seromolecular study to determine the prevalence of cytomegalovirus in pregnant women referred to health centers in the north of Iran

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Because of the controversial aspects of the CMV virus during pregnancy, the present seromolecular study aimed to determine cytomegalovirus prevalence in pregnant women referred to health centers in the north of Iran.

### MATERIALS AND METHODS

One hundred and twenty-five pregnant women who were referred to health centers in Mazandaran province, Iran. To detect the presence of the CMV genome and specific IgM and IgG antibodies against cytomegalovirus, the conventional PCR and ELISA tests were applied respectively.

### RESULTS AND DISCUSSION

The result showed that 2(1.6%), 92(73.6%), and 2(1.6%) of the cases were positive for IgM, IgG, and IgM/IgG, respectively. The PCR test results indicated that the CMV DNA was present in 10 (8%) pregnant women. Our study shows that all PCR-positive cases were negative for the IgM test. Of the 10 PCR-positive samples 3 were positive and 1 was suspicious for the IgG test.

### CONCLUSION

Our study revealed that there is an urgent need for vaccination or other strategies to prevent and treat congenital CMV infection. Reducing the burden of congenital CMV infection requires global awareness. Further studies are recommended to obtain accurate estimates of the risk of congenital CMV infection.

**Keywords:** Seromolecular, cytomegalovirus, pregnant women, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular investigation of identification of virulence genes of *Streptococcus equi* isolated from respiratory samples of foals with acute respiratory infection by multiplex PCR method

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

*Streptococcus equi* is an important pathogenic pathogen in horses, which is associated with respiratory tract infections in foals and is also one of the causes of uterine infections in mares. *Streptococcus equi* can be isolated from various infections in many animals including pigs, sheep, cattle, goats, dogs, foxes, birds, rabbits and guinea pigs. Cases of human infection with this bacterium have also been reported, and such infections are often associated with the consumption of homemade cheese or unpasteurized milk. The aim of this research is to identify the virulence genes (*CspA*, *CspB* and *Spb1*) of *Streptococcus equi* isolated from the respiratory samples of foals with acute respiratory infection by multiplex PCR method.

### MATERIALS AND METHODS

The present study was carried out in a descriptive-cross-sectional manner. It was a research study and the statistical population was among all the horses with upper respiratory infection caused by *Streptococcus equi* (40 cases) in the stables of Kerman province. Diagnostic tests were performed using a long sterile polyacryl swab for sampling from the upper respiratory tract (horse nose). They were immediately transferred to primary culture medium or basic culture medium (Merck-Germany) containing 10% sheep blood (SBA).

### RESULTS AND DISCUSSION

M-PCR reaction was performed on all 40 isolates of *Streptococcus equi* isolated from horses. The results showed that the presence of *CspA*, *CspB* and *Spb1* genes in the isolates under study was confirmed by Multiplex-PCR method. *CspA* and *CspB* genes were present in all isolates (100%), but *Spb1* gene was not found in any of the isolates. From the laboratory samples, *Spb1*, *CspA* and *CspB* genes were identified simultaneously and using specific primer pairs, and *Streptococcus equi* strain producing the desired genes was used as a positive control in all stages. Antibiotic resistance in *Streptococcus equi* is increasing day by day and the situation

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was observed in relation to Tetracycline (R), Streptomycin (R), Penicillin (S), Erythromycin (I).

### CONCLUSION

*Streptococcus* has the ability to produce and secrete super antigens and toxin-producing genes *CspB*, *CspA* and *Spb1*. In this research, identification of *CspB*, *CspA* and *Spb1* genes in *streptococci* (*equi* species) in epithelial cells in tissues infected with bacteria was done by multiplex method. Genetic diversity shows that three genes, *CspB*, *CspA* and *Spb1*, are more effective than other genes in bacterial virulence and virulence, and as an important factor for investigating disease and disease susceptibility, it may also be a potential target for vaccine development in *streptococci*.

**Keywords:** *Streptococcus equi*, virulence genes (*CspA*, *CspB* and *Spb1*), foal respiratory infection, M-PCR (multiplex PCR method).

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### A case study of the treatment strategies employed to cure a COVID-19 patient with multiple infections successfully

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#### ABSTRACT

The COVID-19 pandemic has given rise to new clinical challenges in healthcare settings. One of these challenges includes a heightened risk of secondary invasive bacterial and fungal infections, which have been associated with a notable mortality rate. We report a fatal case of a COVID-19 patient with two bacterial and one fungal infection successfully cured. A woman went to the hospital with fatigue, cough, chest and abdominal pain, nausea, and vomiting symptoms. She tested positive for COVID-19 and had underlying health conditions. She had a bacterial infection called *Klebsiella Pneumoniae*. The bacteria was resistant to many antibiotics, but colistin was effective. After 20 days in the ICU, she developed a fungal and *Enterococcus faecalis* (VRE) infection. The second bacteria was treated with linezolid. After 35 days in the hospital, she was discharged with no signs of infection. Including appropriate bacterial screening and treatment is imperative when dealing with COVID-19.

#### CONCLUSION

Therefore, it is essential to incorporate proper bacterial screening and treatment in managing COVID-19, especially for patients with co-morbidities like diabetes, hypertension and those with indwelling devices.

**Keywords:** COVID-19, *Klebsiella pneumoniae*, *Enterococcus faecalis*, fungal infection, case report.

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# Prevalence and Resistance of Linezolid Resistance Genes (*poxxA* and *optrA*) Detected in *Enterococcus* spp. Isolates from Meat Products in Iran: A Groundbreaking Report

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Enterococcus faecalis* and *Enterococcus faecium* can cause hospital-acquired infections and have the potential to develop resistance to critical antibiotics like linezolid. Understanding the prevalence of resistance genes in non-clinical sources like food products is crucial for monitoring and preventing the spread of multidrug-resistant bacteria.

### MATERIALS AND METHODS

In Isfahan, Iran, 135 meat food samples (chicken, lamb, beef, and ostrich meat) were gathered from various stores. The samples were taken to the Microbiology Laboratory and treated with saline, filtered, and placed on Bile Esculin Agar plates with vancomycin. The colonies suspected to be *Enterococcus* were subjected to identification tests, including biochemical and molecular methods. Antibiotic sensitivity tests were performed according to CLSI 2021 guidelines, and strains resistant to multiple drugs (MDR) were examined for the presence of *cfr*, *poxxA*, and *optrA* genes using multiplex-PCR.

### RESULTS AND DISCUSSION

In study of 135 meat samples, *Enterococci* strains were detected in 59 samples. Among these samples, 69.4% contained *Enterococcus faecalis* (Efa), 15.2% contained *Enterococcus faecium* (Efm), 5.0% contained both Efa and Efm strains, and 10.1% contained other *Enterococcus* strains.

In an antibiotic sensitivity test, tetracycline showed the highest resistance (81.7%), while linezolid had the lowest resistance (9.7%). Two strains of MDR *Enterococci* had the *poxxA* gene, with one strain having only *poxxA* and another strain having both *poxxA* and *optrA* genes. Three strains of MDR *Enterococci* had the *optrA* gene, with two strains having only *optrA* and one strain having both *optrA* and *poxxA* genes. Notably, none of the tested strains had the *cfr* gene.

The statistical analysis revealed a significant correlation ( $P < 0.05$ ) between the presence of *poxxA* and *optrA* genes and resistance to linezolid in multi-drug resistant *Enterococci*.

### CONCLUSION

Increasing the utilization of antibiotics in animal farming and the poultry sector may lead to the proliferation of antibiotic resistance and the transmission of resistance genes to strains found in medical settings. It is crucial to restrict and continuously supervise the usage of antibiotics in non-clinical environments.

**Keywords:** *Enterococcus*, linezolid, Food, *poxxA*, *optrA*

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# Microalgae Exopolysaccharides: Postbiotic with Anti-Adhesive Properties

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The development of antibiotic resistance in *Helicobacter pylori* has emerged as a significant issue in recent times. Using non-biocidal surface-active compounds to prevent bacterial pathogens from adhering and aggregating is a promising alternative to antibiotic treatments. Some anti-adhesive compounds, such as capsular polysaccharides released by different microalgae, have been identified for this purpose. The present study aimed to determine if the sulfated exopolysaccharides extracted from *Chlorella* sp. strain N<sub>4</sub> have any anti-adhesive effects against *H. pylori*.

### MATERIALS AND METHODS

The sulfated exopolysaccharides were extracted using acid (0.07%) at 90°C for 4 hours. The total carbohydrates and sulfate content of exopolysaccharides were analyzed by the phenol-sulfuric acid and BaCl<sub>2</sub> gelatin method respectively. The inhibitory effect of extracted polysaccharides on *H. pylori* attachment to gastric adenocarcinoma (AGS) cell lines was investigated in vitro using AGS-coated microtiter plates.

### RESULTS AND DISCUSSION

The results implied that sulfated exopolysaccharides extracted from *Chlorella* sp. strain N<sub>4</sub> have a 0.41 sulfate/ sugar ratio. The extracted exopolysaccharides could effectively protect AGS cells against *H. pylori*. In addition, a competitive binding assay showed that the antiadhesive activity of exopolysaccharides was higher than heparin. While heparin at the concentration of 250 µg/mL reduced attachment of *H. pylori* to AGS cells by approximately 52.5±3.07%, the extracted polysaccharides caused 71.35±3.47% reduction in adhesion at the same concentration. The observed anti-adhesive properties are likely due to a combination of factors, such as the sulfate content and its binding site, the monosaccharide residues, and the glycoside bonds. These elements are involved in the bioactivity of the polysaccharide.

### CONCLUSION

According to the findings, the sulfated exopolysaccharide extracted from *Chlorella* sp. strain N<sub>4</sub> demonstrated a strong ability to prevent adhesion of *H. pylori* cells in vitro. So, they have remarkable potential in *H. pylori* eradication therapy by inhibition of gastric colonization.

**Keywords:** Exopolysaccharide, microalgae, *H. pylori*, AGS cell.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating the antibacterial effect of Ajwain plant extract on *Aggregatibacter actinomycetemcomitans* bacteria

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Periodontitis is an infectious disease of the tooth - supporting tissue, caused by several microorganisms that make gradual damage to the periodontal Ligament and alveolar bone by creating an envelope, recession, or both. One of the main causes of periodontal disease is *Aggregatibacter actinomycetemcomitans*. Ajwain is a plant with many known properties, including antibacterial. Therefore, the present study was performed to determine the antibacterial effect of this aqueous and alcoholic extract of Ajwain on A.a bacteria.

#### MATERIALS AND METHODS

In this experimental study, the diameter of the growth inhibition zone of aqueous - alcoholic extract of Ajwain on Aa was measured by well plate method on 6 plates and 24 wells with positive and negative control (chlorhexidine 0.2 % and solvent). Finally, the information was entered into Spss software version 22 ( $\alpha = 0.05$ ).

#### RESULTS AND DISCUSSION

The results of the present study showed that the mean diameter of growth inhibition zone for 0.2 % chlorhexidine mouthwash was, Ajwan extract with concentrations of 300 mg/ ml and 150 mg / ml and empty solvent respectively 42.66, 22.5, 16.33 And zero millimeters. Chlorhexidine mouthwash has been shown to have a significantly more growth inhibition zone than two concentrations of Ajwan extract.

#### CONCLUSION

The present study showed that the extract of Ajwain plant has an Antibacterial effect Against A.a and with more extensive studies, this substance may be used in the treatment of periodontitis and other diseases related to A.a.

**Keywords:** Ajwain , Antibacterial , *Aggregatibacter actinomycetemcomitans*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The use of anti-*Helicobacter* effects of herbal compounds to deal with drug resistance

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#### ABSTRACT

*Helicobacter pylori* a spiral bacterium, colonizes in the gastric mucosa of approximately half of the world's population. Its prevalence is different in human and animal species and different geographies and it can be involved in the pathogenesis of gastric ulcer syndrome. The standard treatment of peptic ulcer syndrome includes proton pump inhibitors, bismuth salts, H<sub>2</sub> blockers and antibiotics especially to fight *H. pylori*. Numerous pharmacological studies have been done to eradicate *Helicobacter spp* and there are some major problems in this way. It includes high cost, high global prevalence and resistance to available antibiotics. Therefore, replacing new strategies to prevent or manage *Helicobacter spp* has opened the way to find a natural, accessible and less harmful solution to deal with *Helicobacter*. According to the reviews and research conducted in scientific sources such as Google Scholar, Scopus and WOS, a number of plants that were sometimes consumed traditionally had significant effects on preventing the growth of this bacteria and healing ulcers and inflammation in the stomach, including *canarium album*, *Syzygium aromaticum*, *perfume guava*, *Cuminum cyminum*, *Pimpinella anisum*, *Carum carvi*, *Glycyrrhiza aspera*, *juglans regia*, *Ligustrum vulgare*, *Thymus kotschyanus*, *Trachyspermum copticum*, *Xanthium brasiliense*, *chaemelum nobile*, *cerastium candidissimum*, *origanum majorana*, *stachys betonica*, *fagopyrum esculentum*, *Moringa oleifera*, *green tea*, *zinziber officinale*, *curcuma longa*, *achillea millefolium* and *aloe vera*. The antibacterial effects of some of these plants were comparable to common antibiotics used in the treatment of peptic ulcers, so these plants can be used to produce complementary or even alternative drugs to deal with *Helicobacter pylori*.

**Keywords:** Glanders, *Burkholderia mallei*, melioidosis, horse

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### New findings about the zoonosis disease of GLANDERS

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#### ABSTRACT

Glanders disease is one of the serious and contagious diseases in equines that can be transmitted to humans. The causative agent of the disease is intracellular gram-negative bacterium *Burkholderia mallei*, which was previously known as *Pseudomonas mallei*. Glanders is seen in animals in three forms: cutaneous, nasal and pulmonary. Donkeys suffer from the acute and fatal form, but horses mainly suffer from the sub-acute form of the disease. The risk of bacteria transmission through horses to humans is much more serious. Routine diagnosis of psoriatic arthritis in animals is performed by intradermal test of mallein in the eyelid and complement fixation test. In the searches in scientific sources about the new findings about the glanders, new and practical points about this disease were collected. Unfortunately, suspicious positive cases are still seen in Iran horses, and in a recent study, 1.35% of Tehran and Alborz horses were serologically positive. The infected horse is removed by sanitary methods and the corresponding herd is quarantined. Bacteria are present in nasal secretions and skin lesions and secretions of animals and may be transmitted to humans through contact, inhalation and swallowing. Although the occurrence of glanders in humans is rare, this disease is still reported in Iran in recent years. Recently, new western blood methods and methods based on microbial protein detection with higher sensitivity and specificity than routine methods such as CFT have been used to diagnose the disease. Human cases can cause death if not treated on time. Although in new studies, it has been pointed out that the co-trimoxazole (trimethoprim/sulfamethoxazole) can be useful for the treatment of this disease. Recently, recombinant vaccines have been made for glanders and melioidosis, which can induced significant immunity against bacteria.

**Keywords:** Glanders, *Burkholderia mallei*, melioidosis, horse

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Artemisinin and Leishmaniasis: Insights into Therapeutic Applications

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Leishmaniasis, a chronic disease, has afflicted over 12 million individuals across 98 nations, predominantly within tropical zones. The ailment arises from flagellated protozoa within the *Leishmania* genus, encompassing more than twenty distinct species. These obligate intracellular parasites propagate via mosquito bites. Artemisinin (art), a sesquiterpene possessing an endoperoxide moiety sourced from *Artemisia annua*, stands out for its remarkable effectiveness and minimal toxicity, positioning it as the primary therapeutic agent against parasitic diseases. Therefore, this study delves into Artemisinin's efficacy as a treatment modality for leishmaniasis.

### MATERIALS AND METHODS

In this review study, we investigate English articles from online databases, including PubMed, Scopus, ProQuest, Web of Science, and Google Scholar using “artemisinin”, “Treatment”, and “leishmania” keywords, spanning 2019 to 2023.

### RESULTS AND DISCUSSION

Low-temperature electron paramagnetic resonance (EPR) spectroscopy of *Leishmania* revealed that Art changes the redox state of the sensitive iron pool less than ascaridol EP, questioning its role as a major activator of Atr in *Leishmania*. By inductively coupled plasma methods (ICP-OES, ICP-MS) it was found that these inhibitors do not block iron (heme) accumulation, but are absorbed and act in *Leishmania*. These inhibitors blocked the conversion of hemin to bilirubin in *Leishmania* homogenates, indicating that HO-like enzyme activity exists in *Leishmania*. NADPH-dependent degradation of Art and hemin was highest in the small granule and microsomal fraction of *Leishmania*. EPR spin trapping in the Art/hemin system revealed that NADPH, ascorbate, and cysteine are suitable reductants and ultimately activate Art to acyl-carbon radicals. These findings suggest that it is the main activator of Art in *Leishmania*, both through HO-like enzymatic activities and/or chemical interaction with Art.

### CONCLUSION

Artemisinin-based treatment exhibits potential efficacy in counteracting *Leishmania*'s impact on human health. However, substantiating its effectiveness across various leishmaniasis forms necessitates further in vivo experimentation involving artemisinin and subsequent clinical investigations.

**Keywords: Artemisinin, Leishmania, Treatment**

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# Molecular typing by the TPI gene of *Giardia lamblia* isolates by PCR-RFLP in Khuzestan province

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Giardia lamblia*, also known as *Giardia intestinalis* and *Giardia duodenalis*, is a flagellated parasitic that colonizes the small intestine, causing a diarrheal condition known as giardiasis. Infection with *Giardia lamblia* most often results from fecal-oral transmission or ingestion of contaminated water. *Giardia lamblia* isolates are classified into seven groups based on the characteristics of different genes, including triosephosphate isomerase (TPI). Assemblages A and B infect humans and a broad range of other hosts. The purpose of this study was to genotype human isolates of *Giardia lamblia* by PCR in Khuzestan province.

### MATERIALS AND METHODS

For this purpose, 45 positive fecal samples of *G. lamblia* were collected from 2022 to 2023. DNA extraction and amplification of the TPI gene by nested-PCR successfully were conducted. All samples were positive. To determine the genetic differences, sequencing on three samples was conducted. Then, all PCR products were digested with Bbv1, Mnl1, and RsaI restriction enzymes

### RESULTS AND DISCUSSION

Results showed that the alignment of the TPI sequences obtained with reference sequences indicates the presence of 2 genotypes of *Giardia lamblia* (A and B). The results of the RFLP technique show that 24 of 45 (53.3%) isolates belonged to assemblage A and 21 of 45 (46.6%) belonged to assemblage B.

### CONCLUSION

PCR-RFLP by TPI gene application was able to discriminate between *G. lamblia* assemblage A and B. Also, assemblage A might be an almost dominant genotype in Khuzestan province.

**Keywords:** *Giardia lamblia*, molecular typing, PCR-RFLP, triosephosphate isomerase (TPI) gene, Khuzestan Province, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The prevalence of Beijing and Haarlem genotypes in Iran for *Mycobacterium tuberculosis* with multiple drug resistance: Systematic Review and Meta-Analysis

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

Tuberculosis is caused by the highly virulent respiratory pathogen *Mycobacterium tuberculosis* (MTB) infecting its hosts via aerosol. Resistance to antibiotics is a serious issue that complicates the control of this infection. As a rule, MTB is fastidious and must be diagnosed in laboratories equipped with the necessary equipment. In this regard, molecular methods play an important role in diagnosis and genotyping in a timely manner. A number of multidrug-resistant (MDR) strains of tuberculosis are derived from the Beijing and Haarlem genotypes of MTB. It was the objective of this study to assess the prevalence of Beijing and Haarlem genotypes among MDR-TB cases in Iran using a systematic review and meta-analysis.

### MATERIALS AND METHODS

The current study retrieved 522 original articles (2002-2022) from several databases, such as Medline, Scopus, Embase, Cochrane Library, and Iranian databases. The Beijing and Haarlem families were identified in 22 articles examining the prevalence of MDR tuberculosis strains. A Stata version 17.0 (Stata Corp., College Station, Texas, USA) program was used to evaluate the data.

### RESULTS AND DISCUSSION

In the final investigation, 1152 MDR samples were found in 22 articles. Beijing and Haarlem genotype prevalence were estimated at 19.88% (95% CI, 11.44-29.67) and 15.17% (95% CI, 9.17-22.22) among MDR-TB patients in Iran. It is concerning to note that studies in northern Iran have demonstrated an association between Haarlem genotype and MDR. Considering its potential to endanger Iran's tuberculosis control programs, this genotype poses particular epidemiological and clinical concerns.

### CONCLUSION

In recent years, the Haarlem genotype family has become the most prevalent genotype family in MDR isolates, indicating its importance for TB control. Aside from the high prevalence of this genotype in the south and northeast regions, the prevalence of this genotype has increased by 20.5% in Iran.

**Keywords:** *Mycobacterium tuberculosis*; multi drug resistance; Beijing; Haarlem

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The role of human microbiota in pulmonary tuberculosis

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#### ABSTRACT

Globally, tuberculosis (TB) is not only a serious health issue but is also a major health concern caused by *Mycobacterium tuberculosis*. It is possible that individuals suffering from this disease are not being treated or perhaps are unaware that they are suffering from it. This may be due to the fact that the current treatment, diagnosis, and control strategies for TB may not be as effective as they could be. Dysbiosis of the normal microbiota, as well as epigenetic modifications appear to play a critical role in the pathogenesis of *M. tuberculosis*. While it may be fundamental, there is a lack of understanding about the role microbiota communities and epigenetic mechanisms play in TB infection. The development of experimental microbiota and epigenetics analysis for the purpose of developing innovative and effective TB infection control strategies appears to be a practical way to operate on microbiota and epigenetics analyses. There is also a need to understand how epigenetic mechanisms may be employed in order to design an effective TB control strategy. Therefore, the aim of current study is identifying the correlation between various microbiota communities and epigenetic mechanisms that occur upon TB infection. Such factors can represent a great opportunity for improving its control, while at the same time stressing the necessity for further research and evaluation.

**Keywords:** Epigenetics; Microbiota; *Mycobacterium tuberculosis*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular characterization of Trichomonads isolated from human & animals hosts in Alborz Province

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Trichomonads are amitochondrial anaerobic flagellated protists that are either parasites or commensals, generally living in the digestive or genitourinary tract of humans and animals, which are known to be important agents for gastrointestinal, oral, bronchitis and genital infections in humans and animals. Currently, diagnosis of this organism is made using laboratory methods including direct smear, culture medium and molecular methods. The aim of this study was to determine and identify the types of molecular genotypes of human and domestic trichomoniasis isolates by ITS1 / 5.8S / ITS2 gene in Alborz province.

### MATERIALS AND METHODS

In this study, 81 positive samples of trichomonads isolated from vaginal secretions, oral secretions and genital secretions (preputial sheath) were collected from humans and animals in Karaj region. The samples were examined using wet spreading, TYM media and PCR. Out of the isolated positive cases, 20 samples were sent for sequencing. Based on the observed results, the size of the proliferative product of this gene was 372 base pairs.

### RESULTS AND DISCUSSION

The results of this study were analyzed using phylogenetic tree and neighbor-joining. Furthermore, the genotypes of this family and the possible mutations were investigated in this study.

### CONCLUSION

The results of this study showed that the ITS1/5.8S/ITS2 gene isolated from these samples was more than 99% similar to the sequences in the gene bank. The sequence of the 5/8SrRNA gene and its surrounding areas of ITS1 and ITS2 showed the low polymorphism of this gene. In birds, two genotypes, A and B, in cattle, 'bovine' genotype and in humans, no specific genotype was observed.

**Keywords:** Trichomonads, ITS1/5.8S/ITS2 gene, Phylogenetic analysis, Alborz Province

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# Design and Production of an Engineered Endolysin with Lytic Activity against Methicillin-Resistant *Staphylococcus Aureus*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Improper use of antibiotics has alarmingly led to the emergence of antibiotic resistance. Hence, we urgently needed to find a suitable alternative to traditional antibiotics. Endolysins are enzymes produced at the end of the phage replication cycle and destroy the peptidoglycan of the bacterial cell wall leading to the lysis of the host bacterial cell. These enzymes are species-specific, exhibit high lytic activity, and it is almost impossible for bacteria to develop resistance against them. Lysozyme subfamily 2 (LYZ2) is a modular region of the gene *6l* (*gp6l*) of phage  $\phi$ MR11 with lytic activity against *S. aureus*. However, it does not possess a cell wall recognition domain, usually found in lysins acting against gram-positive bacteria. Therefore, we aimed to design a chimeric endolysin capable of specifically targeting and eliminating methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria.

### MATERIALS AND METHODS

In this study, we engineered the LYZ2 by fusing a *Staphylococcus aureus* cell wall-binding domain (CBD) to its C-terminus and cloned the chimeric protein (named chimeric *staphylococcus aureus*-targeting enzymiobiotic (CST<sub>Enz</sub>)) into the pET28a vector, and expressed the enzyme in *E. coli* BL21 (DE3) cell. The antibacterial property of the enzyme was further evaluated by turbidity reduction assay, disk diffusion assay, and antimicrobial susceptibility testing.

### RESULTS AND DISCUSSION

The engineered lysin displayed a rapid and specific lytic activity against susceptible and Methicillin-resistant staphylococcus aureus and inhibited the growth of the bacteria at concentrations higher than 0.5  $\mu$ g/ml. Besides, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of CST<sub>Enz</sub> were 128 and 64 times lower than those of LYZ2, indicating the increased bacteriolytic activity of the engineered version of the enzyme.

### CONCLUSION

In conclusion, the chimeric enzymiobiotic can be used as a potential antibacterial agent to limit infections caused by methicillin-resistant *Staphylococcus aureus*.

**Keywords:** Endolysin, Methicillin-Resistant *Staphylococcus aureus*, Antibiotic Resistance, Phage, enzyme Engineering

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# Hip Infection Caused by Non-typhoidal *Salmonella* in a Patient with Acute Lymphocytic Leukemia: A case report

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Non-typhoidal *salmonella* can cause infections such as gastroenteritis, enteric fever and bacteremia. But hip infection caused by this microorganism is rare.

### MATERIALS AND METHODS

An 11-year-old girl presented at Shariati hospital's orthopedic department with diagnosis of avascular necrosis (AVN) of the femoral head. Patient also had a history of acute lymphocytic leukemia (ALL). As a part of treatment procedure, surgery was performed. But after that, there was secretions at the site of surgery. Therefore, specimen of patient was sent to the microbiology laboratory and simultaneously antibiotic therapy with meropenem and vancomycin was administered. The levels of C-reactive protein and ESR were elevated. In microbiology laboratory, biochemical tests was conducted and according that the isolated bacteria was citrate positive, urease negative, H<sub>2</sub>S positive, indole negative, motility positive, lysine decarboxylase positive, ornithine decarboxylase positive, ortho-Nitrophenyl-β-galactoside (ONPG) negative, methyl-red positive and had an alkaline/acid (K/A) reaction on triple sugar iron agar (TSI). Parallel to these tests, serogrouping test was done and was concordance with biochemical tests. Also, Kirby Bauer disc diffusion test was performed and bacteria was susceptible to cotrimoxazole and ampicillin. For confirmatory diagnosis isolated colony was submitted to VITEK® 2 (bioMérieux, Marcy L'Étoile, France). The output result was non-typhoidal *Salmonella*. After successful treatment the patient was discharged from hospital.

### CONCLUSION

Our case report study indicated that non-typhoidal *salmonella* can be isolated from hip infection, so the laboratory technicians must be alert and consider possible present of these bacteria in such infections.

**Keywords:** *Salmonella*, Hip infection, VITEK

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The prevalence, antibiotic resistance rates, and virulence determinants of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from children with cystic fibrosis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Cystic fibrosis (CF) is a lethal genetic disease caused by a mutation in the CFTR gene. It affects the mucosal surfaces and results in the thickening of the mucus of airways, which leads to lung failure and makes an appropriate condition for the growth of microorganisms and respiratory infections. Recently, CF infections with methicillin-resistant *Staphylococcus aureus* (MRSA) have increased extensively in children. The aim of the present study was to evaluate the prevalence, antibiotic resistance rates and frequency of the virulence factors e.g. Biofilm, alpha-hemolysin and penton-valentine leukocidin of MRSA isolated from CF children.

### MATERIALS AND METHODS

In total, 88 sputum samples were collected from children with cystic fibrosis admitted to the Children's Medical Center Hospital from June to April 2023 in Tehran, Iran. The typical phenotypic and biochemical tests were used to validate the isolates' identities. MRSA strains were detected using the cefoxitin (30 µg) disk agar diffusion (DAD) test, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Resistance of isolates against doxycycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), clindamycin (2 µg), and linezolid (30 µg) were assessed by DAD test. The genes encoding virulence factors, such as alpha-hemolysin (*hla*) and penton-valentine leukocidin (*pvl*) were detected by PCR method. The crystal violet assay was utilized to evaluate the biofilm formation by isolates.

### RESULTS AND DISCUSSION

The frequency of *S. aureus* isolates from CF patients was 47% , among which 30 isolates (73.17%) were resistant to cefoxitin (MRSA). The highest and lowest resistance rates were against trimethoprim-sulfamethoxazole (49%) and linezolid (19%), respectively. The *pvl* encoding gene was detected in one isolate (2.5%), while *hla* gene was found in 35 (79%) *S. aureus* isolates. Nineteen isolates (47%) produced strong biofilm.

### CONCLUSION

According to the data, it can be concluded that MRSA is a prevalent pathogen in CF patients. Its resistance against current antibiotics and ability to produce virulence factors like biofilm, alpha-hemolysin, and leukocidin penton valentin can deteriorate the status of the infection. The monitoring of MRSA infection may reduce the mortality rate in early ages of CF patients.

**Keywords:** Cystic Fibrosis, *Staphylococcus aureus*, biofilm, antibiotic resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Phosphorus availability and sugarcane yield in a calcareous soil in response to plant growth-promoting rhizobacteria (PGPR) inoculation

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Low phosphorus availability is a significant challenge in calcareous soils that are cropped to sugarcane in Khuzestan province in Iran. Although in calcareous soils the total P content may be relatively large, a major problem is the low availability of P due to the high content of calcium carbonate, which is reducing P uptake by plants. Among alternatives to overcome this limitation is the use of plant growth-promoting rhizobacteria (PGPR) that form symbiotic associations with most land plants. PGPR can improve the P availability to plant and plant yield through release of organic acids and producing numerous plant growth regulators. Therefore, the objective of this study was to evaluate the effect of inoculation with selected PGPR on P availability and sugarcane (*Saccharum officinarum* L.) yield in a calcareous soil located in the Debal Khozaei sugarcane agro-industry.

### MATERIALS AND METHODS

The experimental design was a randomized complete-block design with three replicates. Treatments consisted of 1. Control treatment (without inoculation), 2. *Enterobacter cloacae* R33 and 3. A mixture of PGPR including *Enterobacter cloacae* R33, *Staphylococcus hominis* 9E and *Brevundimonas* sp. Treatments were applied in second ratoon sugarcane (CP73-21 cultivar) using soil spray method. Population of these bacteria was  $1.7 \times 10^7$  CFU mL<sup>-1</sup> (CFU, colony forming unit). At the end of growth period, the soil available P concentration, shoot P uptake, height and cane yield were measured.

### RESULTS AND DISCUSSION

The results indicated that soil available P and plant P uptake increased significantly ( $P < 0.05$ ) in both PGPR treatments when compared with the control. The inoculation of *Enterobacter cloacae* R33 significantly ( $P < 0.05$ ) increased cane height and yield compared to the control (no PGPR). Comparison of PGPR treatments indicated that higher cane height and yield were obtained for *Enterobacter cloacae* R33 inoculation.

### CONCLUSION

It could be concluded that inoculation of *Enterobacter cloacae* R33 to calcareous soils with low P availability may have beneficial effects on soil P uptake by sugarcane and can potentially be an effective strategy to improve ratoon sugarcane (CP73-21 cultivar) yield.

**Keywords:** *Enterobacter cloacae* R33, Microbial inoculation, Phosphorous uptake, Ratoon sugarcane

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### ***In-vitro* activity of Antimicrobial Agents Combination against carbapenem nonsusceptible *Pseudomonas aeruginosa***

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#### **ABSTRACT**

#### **BACKGROUND AND OBJECTIVES**

Current study was aimed to explore *in-vitro* antimicrobial combinations effects against planktonic and biofilm forms of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA).

#### **MATERIALS AND METHODS**

Twenty-four CRPA were isolated from different clinical specimens. The resistance to carbapenem were determined by the disk diffusion assay and PCR. The antimicrobial effect of antimicrobial agents was determined by the broth micro dilution according to the CLSI guideline. To study the inhibitory against the biofilm, the minimum biofilm inhibitory concentration (MBIC) was determined. The synergetic effects of the drugs combinations, the checkerboard assay was used for fractional inhibitory concentration (FIC) determination.

#### **RESULTS AND DISCUSSION**

The highest synergic interaction was observed in colistin/fosfomycin and gentamicin/fosfomycin (18 of 24 isolates), and the lowest synergic interaction was observed in gentamicin/imipenem and colistin/gentamicin (5 of 24 isolates). Colistin/fosfomycin, imipenem/fosfomycin, colistin/imipenem, gentamicin/fosfomycin, and gentamicin/imipenem were shown synergic effect for 8, 4, 4, 3 and 3 isolates, respectively.

#### **CONCLUSION**

The combination of antimicrobial agents had unlike activity on the biofilm and planktonic forms of CRPA. Thus, a distinct testing of inhibitory activity of the antimicrobial agent's combination is required. Fosfomycin/colistin and fosfomycin/gentamicin were more effective against planktonic form and fosfomycin/colistin against biofilm forms.

**Keywords:** Biofilm, Antimicrobial Combination, Carbapenem-resistant, *Pseudomonas aeruginosa*, wound

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Antimicrobial combinations against drug-resistant *Acinetobacter baumannii*: an *in-vitro* study

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

This study was aimed to explore effect of antimicrobial drugs combination against drug-resistant *Acinetobacter baumannii*.

#### MATERIALS AND METHODS

Twenty-four *A. baumannii* isolates were obtained from different clinical specimens included wounds, UTIs, sepsis and respiratory tract infections during 2022-2023, Tabriz, Iran. The resistance to antimicrobial agents were determined by the disk diffusion and microbroth dilution methods according to the CLSI guideline. Synergetic effects of the drugs combinations were determined using the checkerboard assay was used for fractional inhibitory concentration (FIC) index calculation.

#### RESULTS AND DISCUSSION

The highest synergic interaction was observed in colistin/imipenem and gentamicin/imipenem (17 of 24 isolates), and the lowest synergic interaction was observed in colistin/gentamicin and colistin/ciprofloxacin (6 of 20 isolates). Antagonist effects were not observed.

#### CONCLUSION

Distinct and standard testing of inhibitory activity of the antimicrobial agent's combination is required for treatment of high-level resistant pathogens. Colistin/imipenem and gentamicin/imipenem were more effective against carbapenem resistant *A. baumannii*.

**Keywords:** *Acinetobacter baumannii*, Antimicrobial Combination, resistant

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Molecular typing of the small-subunit ribosomal RNA of *Blastocystis hominis* isolates by PCR-RFLP in Alborz Province

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Blastocystis* sp. is an enteric protozoan that frequently colonizes humans and many animals. and it is involved in the development of gastrointestinal disorders. Despite impacting on human health, data on the prevalence and subtype (ST) distribution of *Blastocystis* sp. remain scarce in Iran, and poor study performed in Alborz Province (Karaj).

#### MATERIALS AND METHODS

320 stool samples were obtained from February and December 2022. DNA of the samples was extracted using the CTAB (Cetyltrimethylammonium bromide) method and Nested PCR using *SRIF*, *SR1R*, *BLF1* and *BLR2* primers and standard strains were optimized and carried out on the samples, then RFLP technique using *Alu I*, *Hinf I* and *Rsa I* enzymes was performed to *blastocystis*.

#### RESULTS AND DISCUSSION

The results of PCR using primers showed the amplification of 1100bp segment and *Blastocystis* contamination in 50 out of 320 tested samples. Our data identified two different *Blastocystis* STs (ST1-ST3) in 44 samples (34= ST1 10= ST3). However, six samples remained undefined. Based on the current findings, ST1 was the most frequent subtype among all positive samples.

#### CONCLUSION

The current study investigated the prevalence and genotypes of *Blastocystis* sp. in individuals who were referred to medical laboratories in Karaj, Iran. Having a better understanding of *Blastocystis* sp. subtype distribution and risk factors would lead to improved preventive measures. Also the necessary of using other sets of primers and designing new primers to *blastocystis* in Iranian isolates

**Keywords:** *Blastocystis*, subtypes, SSU-rDNA, PCR-RFLP, Alborz, Iran,

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Molecular assessment of recreational water contamination with adenovirus

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Human adenoviruses* belong to the *Adenoviridae* family and include more than 100 types of *adenoviruses*. which are divided into seven types based on their biological and biochemical characteristics. They have human diseases, respiratory injuries, gastroenteritis, eye injuries (pictured eye), mango encephalitis and hemorrhagic bladder. A variety of chemical, chemical, and biological contaminants can reduce water quality. Pathogens in water, including viruses, protozoa, pathogens, and a small number of fungi, cause disease. are public While about 150 viruses from water are related to human diseases. *Human adenoviruses* due to their abundant environmental and relative resistance throughout the year, cytopathic development in cultured cells, their appearance similarity to human pathogenic viruses such as *rotavirus* and *norovirus* and lower risk for patients. They are considered as a suitable model for the evaluation of water environmental virus.

#### MATERIALS AND METHODS

Research method: samples of recreational waters such as swimming pools, rivers, seas, etc. were collected, and water samples were filtered using 0.22 and 0.45 paper filters, and the general *adenovirus* primer was designed. Viruses were used by polymerase chain reaction (PCR).

#### RESULTS AND DISCUSSION

7 samples out of 60 collected samples were infected with *adenovirus*.

#### CONCLUSION

The purpose of this study is to investigate the molecular contamination of recreational waters with *adenovirus*. Considering the resistance of gastrointestinal viruses such as *adenovirus* to the purification process, there is a need to pay more attention to the evaluation and control of these viruses

**Keywords:** *Human adenoviruses*, recreational waters, gastrointestinal, paper filters, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating antibacterial properties of nanochitosan/pectin film containing aqueous, aqueous-alcoholic and alcoholic extracts of *Rubia tinctorum* root on some bacterial food-borne pathogen

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Progress in research and development of new technologies related to the production of active food packaging has been noted in recent years. Chitosan can be obtained mainly through the deacetylation of chitin with NaOH. Deacetylation of chitosan not only improves solubility in acidic environment, but also increases antibacterial activity. Pectin is one of the most important renewable natural polymers that is ubiquitous in nature. Pour casting method is the most used technique to obtain pectin based films. *Rubia tinctorum* (*Rubiaceae*), commonly known as Ronas madder root, grows naturally in the Central Asia. In addition, its high efficiency has been seen not only in medicine, but also in the treatment of pathogens. Food-borne diseases caused by microorganisms is growing public health problem via consuming contaminated foods. These pathogens include Salmonella, Staphylococcus aureus, and bacillus cereus, Escherichia coli and other pathogens.

#### MATERIALS AND METHODS

prepare Ronas root extract Extraction from water, ethanol, aqueous-ethanolic, methanol and aqueous-methanol solvents were used. Pectin and chitosan nanocomposite films were prepared. Minimum inhibitory concentration (MIC) tests were done on four bacteria. Circular discs of nano chitosan/pectin films containing different form extraction of Ronas root extract were placed on bacterial with special ATCC lawn.

#### RESULTS AND DISCUSSION

The result of this study showed Incorporation of nano chitosan/pectin films containing different *Ronas root* from ethanolic and methanolic extraction showed significant antimicrobial effects on Staphylococcus aureus ( $P < 0.05$ ). and recommended for food packaging. Mashak et al. (2015) evaluated the antimicrobial effect of chitosan films containing lavender essential oil on some food-borne bacteria. The results indicated the antimicrobial effect of chitosan films containing essential oil on the tested microorganisms and chitosan films containing 4% essential oil All microorganisms were effective.

**Keywords:**Antibacterial Properties , Nanochitosan/Pectin Film , Rubia Tinctorum Root , Bacterial Food-Borne Pathogen

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Molecular investigation of the contamination of some recreational waters in Mazandaran province with *BK* and *JC* viruses

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Water is considered as the most vital component in the origin of life. Water can be contaminated by various industrial toxic and inorganic pollutants as well as pathogens. Contaminated water is responsible for the transmission of various diseases and is considered the main cause of death of approximately 502,000 people worldwide each year. Viruses cause 10 to 10,000 times more disease risk than bacteria. *Human polyomaviruses JCV* and *BKV* are good candidates because they are commonly found in environmental water samples from different geographical regions with relatively high abundance. *Polyomaviruses* are excreted in urine and can spread through water.

#### MATERIALS AND METHODS

This research was conducted on 60 samples of recreational water in Mazandaran province (pool, pond, river, sea). *Human polyomaviruses JCV* and *BKV* were isolated by filtration method using filters (0.22 and 0.45). Specific primers were designed for *BKV* and *JCV*, and the molecular method of polymerase chain reaction (PCR) were detected.

#### RESULTS AND DISCUSSION

*Human polyoma virus JCV* and *BKV* in recreational water samples based on 60 analyzed samples, *JCV* was detected in 23 cases (38.33%), but *BKV* was not observed.

#### CONCLUSION

Due to the high prevalence of viral diseases caused by contaminated water and due to the importance of this issue, which will lead to serious risks if health principles are not followed, the presence of *BK* and *JC* virus in recreational waters was investigated.

**Keywords:** Contamination, recreational waters, *BKV*, *JCV*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Structural analysis of Omicron mutated variant in SARS-COV-2 spike protein

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Since the beginning of the 21st century, coronaviruses of animal origin have been the cause of several deadly pneumonia epidemics in humans. These include SARS, MERS, and currently, Covid-19, which has spread throughout the world as a pandemic. All viruses, including SARS-CoV-2, change over time. Although most of the changes do not have much of an effect on the properties of the virus, but some changes may affect their properties. The spread of Omicron (BA.1), a new type of SARS-CoV-2, has raised serious concerns due to the large number of mutations in its genome and the lack of knowledge about how these mutations will affect the current SARS-CoV-2 vaccines and treatments. Investigating the effects of mutations in the RBD (Receptor Binding Domain) and NTD (N-terminal Domain) domains of the spike protein is important for the development of COVID-19 vaccines.

### MATERIALS AND METHODS

In this study, the structures were modeled using EasyModel, and molecular docking was performed using ClusPro. Computational methods were then used to study the structural effect of mutations in the RBD and NTD regions. Binding affinities were quantitatively calculated using Molecular Dynamics Simulations and free energy calculations.

### RESULTS AND DISCUSSION

Calculations of the binding free energy using the MM/PBSA (Molecular Mechanics Poisson-Boltzmann Surface Area) method show that Omicron binds to the ACE2 (Angiotensin-converting enzyme 2) receptor with a higher binding energy than the wild type, and also has a higher level of contamination compared to the wild type. Furthermore, the results show that the mutations in the NTD and RBD regions show significant differences from the wild type and can reduce recognition by antibodies, resulting in potential immune evasion and a decrease in the effectiveness of existing vaccines.

### CONCLUSION

This study provides a better understanding of the structural changes of the Omicron variant compared to wild type SARS-CoV-2 for planning new treatments and vaccine candidates against this variant of the virus. Nevertheless, it is mainly limited to structural predictions and these findings require further investigations and experiments in order to be confirmed.

**Keywords:** Coronavirus, Omicron variant, Structural analysis, Molecular dynamics simulation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# PCRC: Avoiding Cross-Reactivity in Real-Time PCR Diagnostic Kits

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Introduction: Diagnostic kits that utilize Real-Time PCR technology allow for identifying pathogenic microorganisms by detecting a portion of their genome. Specific primers and probes are designed for the target microorganism's genome to achieve this. During PCR, these primers amplify the target genome if present in the sample. The specificity of these primers and probes is crucial to avoid cross-reactivity and prevent the production of PCR products from other pathogens. When designing and producing diagnostic kits, *in silico* checks for potential cross-reactivity with other pathogens must be performed.

### MATERIALS AND METHODS

In this study, we developed a web-based tool called PCRC (Primer Cross-Reactivity Check) using Python programming language and bioinformatics algorithms. PCRC is used to determine the specificity of primers and probes designed to detect target microorganisms relative to the genomes of other pathogenic microorganisms.

### RESULTS AND DISCUSSION

In this project, we were able to use the PCRC web application to review the results of probes and primers designed to identify pathogenic microorganisms and see the results indicating false positives.

### CONCLUSION

PCRC is a web-based program that performs cross-reaction tests by selecting the desired organisms, allowing for the elimination of false positive results in PCR tests.

**Keywords:** Real-Time PCR, diagnostic kits, primers, probes, cross-reactivity

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### A novel peptide-conjugated gold nanoparticles with potent antibacterial and antibiofilm activity against methicillin-resistant *Staphylococcus aureus* (MRSA)

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Regarding the rapid increase of antibiotic resistance and the defeat of antibiotics to treat biofilm-associated infections, it is urgent to develop new therapeutic tactics. Antimicrobial peptides (AMPs) are one of the promising molecules for fighting bacterial infections because they target bacterial membranes. Although, AMPs have some impediments hindering their further development including high manufacturing costs, low stability, and penetrability. Therefore, several types of research conducted to improve their efficacy and reduce their limitations, including the conjugation of peptides to nanoparticles. In the current work, we suggested a new core-shell formulation with a gold nanoparticle (GNP) core and a hydrophilic cationic peptide surface. We used an J1 peptide, to establish a covalent link and concentrate the peptide on the surface of the gold nanoparticle.

#### MATERIALS AND METHODS

Methicillin-resistant *Staphylococcus aureus* (MRSA ATCC43300) and one clinical MRSA strain, which forms a strong biofilm, were included in this study. GNPs have been synthesized using the wet chemical route, and the conjugate was prepared by mixing different amounts of peptide aqueous solution with the gold nanoparticle solution. Synthesized samples were characterized with UV-Visible, transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), Zeta potential. The minimum inhibitory concentrations (MICs) of formulations were determined by broth microdilution susceptibility test. Biofilm formation after formulations treatment was evaluated by crystal violet assay.

#### RESULTS AND DISCUSSION

The average sizes of GNPs were about 6 nm. Zeta potential of GNPs showed a negative surface charge ( $-7$  mV), and the conjugate was positively charged ( $+7$  mV). FTIR analyses indicated that the peptide successfully conjugated onto GNPs. Comparing the free peptide or GNPs alone, the conjugated variant, has three times higher antibacterial activity (MIC and MBC of conjugate =  $125 \mu\text{M}$ ). The results indicated that conjugate shows improved antibiofilm activity than the peptide and GNPs (minimum biofilm inhibitory concentrations (MBIC) =  $250 \mu\text{M}$ ). The enhanced antibacterial and anti-biofilm activity of the conjugated formulation could be attributed to the increased number of AMPs per NP, resulting in an improved local positive charge density, ready to break down into bacterial cells and efficiently penetrate the biofilm layer.

#### CONCLUSION

The current study has effectively shown that AMP conjugated NPs could be a useful approach for boosting peptide's antibacterial and antibiofilm properties.

**Keywords:** Antimicrobial peptide, Gold nanoparticle, Antimicrobial activity, Antibiofilm

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Phenotypic detection of efflux pump in *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates from burn wound infection

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Some bacteria showed resistance to beta-lactamase after some time. Efflux pumps play a very important role in creating antibiotic resistance of *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains. This study aims to investigate microbial resistance and phenotypic investigation of efflux pump in *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains isolated from burn patients. Recent studies suggest that efflux pumps may be used by the cell as a first-line defense mechanism to avoid lethal drug concentrations until.

### MATERIALS AND METHODS

Phenotypic investigation of efflux pump by Cardwell method: In order to investigate the activity of efflux pump phenotypically, all isolates were investigated using agar technique containing ethidium bromide by Cardwell method.

### RESULTS AND DISCUSSION

In the present study, 20 wound samples of burn patients were infected with *Pseudomonas aeruginosa* and 10 samples were infected with *Staphylococcus aureus*. *Staphylococcus aureus* was resistant to ampicillin, penicillin and cefazolin 100% and cefotaxime, levofloxacin, ciprofloxacin, 70%, 80%, 90%, respectively, and *Pseudomonas aeruginosa* was resistant to imipenem, ciprofloxacin, levofloxacin, ceftazidime, 85%, respectively. 65%, 55%, 85% were resistant. 60% *Pseudomonas aeruginosa* produced ESBL, 70% *Staphylococcus aureus* produced beta-lactamase. 75% and 70% of MDR isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* had efflux pumps, respectively.

### CONCLUSION

The findings indicate that most of the isolates collected from patients with severe burns include strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and most isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed multidrug resistance and produced beta-lactamase enzymes. It was also shown that most of the isolated bacterial population with multi-drug resistance and beta-lactamase enzyme production have an efflux pump, so it is important to investigate the presence of this pump in proposing a suitable treatment model for patients infected with these bacteria. Considering the presence of the efflux pump in *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains, it is important to investigate the presence of these genes in proposing a suitable treatment model for patients infected with these bacteria in medical centers.

**Keywords:** Antibiotic Resistance, *Pseudomonas Aeruginosa*, *Staphylococcus Aureus*, Efflux Pump

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The frequency and antibiotic resistance patterns of Livestock-associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) isolated from bovine-mastitis in Iran, a retrospective study

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Bovine mastitis is an influential zoonotic infection that impacts cows' health and decreases the production yield of milk and dairy products. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important causes of bovine mastitis, which makes the treatment challenging, and its transmission to humans through the consumption of infected meat or milk is very concerning. This study aimed to determine the frequency and antibiotic resistance patterns of MRSA isolated from milk samples of bovine mastitis in three provinces of Iran.

### MATERIALS AND METHODS

A total of 242 isolates of *S. aureus* recovered from milk samples of bovine mastitis were collected from previous studies performed from 2010 to 2020 in Tehran, Mashhad, and Hamadan provinces of Iran. The identity of the isolates was confirmed using the standard phenotypic and biochemical tests. MRSA strains were detected using the cefoxitin (30 µg) disk agar diffusion (DAD) test, according to the guidelines of Clinical and Laboratory Standards Institute (CLSI). Subsequently, PCR of the *mecA* gene was applied to confirm MRSA isolates. Resistance of isolates against doxycycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), clindamycin (2 µg), cefazolin (30 µg), and linezolid (30 µg) were assessed by DAD test.

### RESULTS AND DISCUSSION

The identity of 147 isolates was confirmed as *S. aureus*, among which 34 isolates (23.12%) were resistant to methicillin (MRSA). The *mecA* gene was detected in 94.11% of isolates (N= 32/ 34). The frequency of MRSA in Hamadan, Tehran, and Mashhad was 68%, 32.35%, and 0%, respectively. Isolates showed high resistance against cefazolin (85%) and clindamycin (79%) and had low resistance to trimethoprim-sulfamethoxazole (35 %) and doxycycline (3%), respectively. All isolates were susceptible to linezolid.

### CONCLUSION

This study demonstrates that the frequency of MRSA in bovine mastitis in Iran is not low and should not be underestimated. In addition, the results of antibiotic sensitivity tests indicate the need to check the antibiotic resistance of the isolates before selecting the appropriate treatment strategy. Considering the importance of transmitting this pathogen from livestock products to the human population and its high-level resistance against most first-line antibiotic treatment regimens, it is necessary to conduct periodic studies to determine the accurate prevalence of LA-MRSA and its antibiotic resistance patterns throughout Iran.

**Keywords:** Bovine mastitis, *Staphylococcus aureus*, LA-MRSA, antibiotic resistance, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# In silico design of ferritin-EBV gp350 nanoparticle vaccine

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The Epstein-Barr virus (EBV) is related to the emergence of different malignancies such as Infectious Mononucleosis (IM) and epithelial cell malignancies. The prevention of EBV infection and the management of EBV-related diseases have been proposed to be achievable through prophylactic and therapeutic vaccination. For the past two decades, researchers have studied the major envelope protein gp350 in EBV vaccinations. This protein attaches to B cells through complement receptor 2 (CR2/CD21). Despite years of research, there is no available licensed vaccine for EBV, one of the latest attempts in this field is the ferritin nanoparticle vaccine designed by Kanekiyo et al. In this study, a ferritin-based nanoparticle vaccine that represents gp350 was computationally designed.

### MATERIALS AND METHODS

The structure of gp350 protein "2h6o" was selected from PDB, and the structure of ferritin protein is sourced from Masoomi et al. HADDOCK 2.4 server was employed for the molecular docking of ferritin-gp350. PyMol molecular viewer was used to examine the clusters.

### RESULTS AND DISCUSSION

Molecular docking results are shown in 10 clusters. The cluster that had the best positioning of gp350 on ferritin, along with a low RMSD (0.7 +/- 0.5) and HADDOCK score (-14.1 +/- 18.8), Electrostatic energy (-488.7 +/- 67.2), Z- score (-2.4) is presented here as the top molecular docking outcome.

### CONCLUSION

In this study, a structure was chosen, which is the best molecular docking structure of the gp350 protein, which is located in the vertical position of ferritin and has better accessibility for CR2. The binding sites of gp350 to ferritin were investigated and it was shown that the sites required for binding of gp350 to CR2 protein are free for binding, which facilitates antigen presentation and self-assembly of ferritin nanocages.

**Keywords:** EBV -nanoparticle vaccine- gp350 - ferritin-

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Optimization of suitable carbon source for polysaccharide production by *Lactobacillus* sp. UTMC 3983 and molecular identification of the producer strain (EPS)

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Currently, exopolysaccharide is one of the most important and widely used biopolymers with many industrial uses in industries such as pharmaceutical, food, agriculture, etc. Lactic acid bacteria are biological factories that produce these biopolymers. With the increasing demand in the use of these biopolymers, the commercialization of the products of these bacteria and their replacement with expensive and rare products can facilitate many industrial processes and make the final product more economical. For this reason, newly isolated strains with the ability to produce more exopolysaccharide such as *Limosilactobacillus* using optimal culture medium have become one of the attractive fields of industrial microbiology research. The aim of this study was to propose different sources of carbon, as well as examining different concentrations of these sources, to find the optimal carbon source and the appropriate concentration for exopolysaccharide production in order to optimize the best conditions for increasing the production of lactic acid producing bacteria.

### MATERIALS AND METHODS

In this study, the lactic acid strain of *Lactobacillus* sp. UTMC 3983 from the Microbial Collection Center of the Division of Microbiology (University of Tehran) was used and regenerated on MRS Broth culture medium. After ensuring the purity of the colonies, bacteria were inoculated on the Erlenmeyer flask containing the MRS broth culture medium (30 °C, 180 rpm for 24 hours). Then, 10% of the volume of pre-cultivation flasks was replaced with other carbon sources (including lactose, whey, molasses, glucose syrup, and sucrose) in Erlenmeyer flasks containing production medium based on MRS broth and polysaccharide production, residual substrate and bacterial biomass are measured. In order to sequencing of the strain, DNA extraction and RT-PCR technique were performed and the obtained sequences were aligned with the related sequences from NCBI and other sequences outside the genus.

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Finally, MEGA software and Maximum Parsimony criterion were used to construct a phylogeny tree and evolutionary genetics analysis.

### RESULTS AND DISCUSSION

By examining the addition of 2.5% to 60% concentrations of sucrose as a carbon source to the culture medium and examining the amount of changes in exopolysaccharide production, it was determined that the highest production rate was at a concentration of 30%, 67 g/liter, which was used as a control medium in the efficiency of exopolysaccharide production from different carbon sources. By replacing the concentrations of (20-25-30-35-40) % whey as a carbon source, the amount of exopolysaccharide production in the highest concentration of whey (40%) was obtained less than a third of the amount of exopolysaccharide production in control medium. Also, by replacing the concentrations of (30-40-50-60) % lactose as a carbon source, the amount of exopolysaccharide production at concentrations of 30% and 40% was reported to be very insignificant and about zero, and concentrations of 50% and 60% represented lactose sediment, which caused the loss of the ability to the little exopolysaccharide production. By replacing the concentrations of (10-20-30-40)% of the glucose syrup, the average amount of exopolysaccharide production in the medium with the highest concentration (40%) was detected as 24.4 g/liter, which is about half of the amount of production exopolysaccharide was optimized in control medium; However, exopolysaccharide production in the presence of beet molasses with concentrations of (20-30-40)% in the culture medium was not detectable and the beet molasses remained unused as sediment at the end of the test process. Optimizing the production medium in terms of pH and time and conditions of bacterial growth showed that the amount of bacterial turbidity in the medium with pH=5.7 was higher (average: 2.212) compared to the medium with pH=6.8 on average (average: 1.994). The growth of bacteria was reported to increase in the first 48 hours, and the shape and size of *Limosilactobacillus* was directly related to the amount of exopolysaccharide production.

### CONCLUSION

The results of this study showed that the bacteria were unable to use lactose sugar, either in pure form or in whey, and beetroot dremelas could not grow. The results of this study showed that the bacteria could not use lactose sugar, either in pure form or in whey, and can not grow in the presence of beetroot. By examining glucose syrup as a carbon source, it was also determined that this carbon source is only suitable for the bacteria revival, although exopolysaccharide production in this medium was weak. Finally, the medium with 30% sucrose (approximately 67 g/L) was found to be the most suitable carbon source for the growth and production of exopolysaccharide. Our findings showed that increasing the pH of the medium as well as increasing the fermentation time did not show a positive effect on the amount of exopolysaccharide production.

**Keywords: Optimization, Exopolysaccharide, Lactic acid bacteria, Limosilactobacillus.**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The relationship between vaginal infections and gestational diabetes

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Gestational diabetes is defined as glucose intolerance with variable severity which starts or is first diagnosed during pregnancy. Globally on the rise, this health condition is one of the most common complications of pregnancy. Gestational diabetes increases the probability of some complications in the mother and the fetus during and after pregnancy, including preeclampsia, polyhydramnios, fetal macrosomia, difficult delivery, metabolic complications in the infant (hypoglycemia, hyperbilirubinemia, hypoglycemia, and hypotonia), and perinatal mortality. This study aimed to examine the relationship between vaginal infections and gestational diabetes.

#### MATERIALS AND METHODS

This study enrolled 300 pregnant women with gestational diabetes as the patient group and 300 pregnant women without gestational diabetes as the control group. The research tool in this study was a questionnaire and performing a warm and wet slide staining test on the vaginal swab sample.

#### RESULTS AND DISCUSSION

The mean age was  $31.97 \pm 6.02$  in the women with gestational diabetes and  $30.98 \pm 6.80$  in the women without gestational diabetes. Smoking was zero in both groups. The frequency of vaginal infection was 34(11.3%) in the patient group and 26(8.7%) in the control group.

#### CONCLUSION

Despite its high prevalence, vaginal infections showed no significant association with gestational diabetes. Still, the high prevalence of infection in the two groups demands more attention from the healthcare system and obstetrician-gynecologists to check the infection before and after pregnancy.

**Keywords:** Gestational diabetes, Vaginal infection, Pregnancy, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Optimization of fibrinolysin production by *Alcaligenes faecalis* strain 7

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Fibrin is produced during blood clotting which can cause thrombosis and consequently myocardial infarction and cardiovascular diseases. Fibrinolysin is the enzyme responsible for fibrin clot digestion. The aim of this study was to optimize fibrinolysin production by *Alcaligenes faecalis* strain 7.

### MATERIALS AND METHODS

The isolate was cultured in Nutrient broth and incubated at 30, 37 and 43°C and the optimum temperature was obtained based on the growth curve analysis. Subsequently, it was cultured in Nutrient broth at pH 6, 7 and 8 and incubated at optimum temperature from previous step for 24h and optimum pH was obtained. Following bacterial culture at optimum pH and temperature, sterile blank discs were saturated with the supernatant of isolates (10000 rpm, 5 min) and placed for 24h at 37°C on plasma plate (1ml of plasma, 4ml agar and 1ml of thromboplastin D) and the amount of fibrin digestion was determined based on the diameter of formed halo zone.

### RESULTS AND DISCUSSION

As a result of this study, optimum temperature and pH for *Alcaligenes faecalis* strain 7 were obtained as 30°C and 6, respectively. In these conditions it was led to an increase in fibrinolysin production which was identified with 11mm clear halo zone

### CONCLUSION

Based on the obtained results it can be concluded that for *Alcaligenes faecalis* strain 7 can efficiently produce fibrinolysin at its optimum temperature and pH which can be used for scale up production of this enzyme by this isolate.

**Keywords:** *Alcaligenes faecalis* strain 7, Fibrinolysin, Plasma plate

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the effects of postbiotic mixture (*Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus heloticus*) on the antioxidant factors of testicular tissue infected with *Escherichia coli* bacteria in rat

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Probiotics have several properties depending on the strain. Some probiotics play an important role in preventing infection and balancing the immune system due to the interaction between the intestinal mucosa and cells in the immune system. This study aims to investigate the properties of probiotic mixture (*Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus heloticus*) on the antioxidant factors of testis tissue infected with *Escherichia coli* bacteria in rats.

### MATERIALS AND METHODS

In this experimental study, 21 animals were divided into three groups, including the control group, infected with *Escherichia coli* bacteria ( $10^8$  CFU/ml) and the infected model + receiving probiotics ( $10^9$  CFU/ml). Induction of infection was done by intraperitoneal injection and receiving probiotics for 35 days by gavage method. After the treatment and dissection of the animals, the testis tissue was extracted to evaluate the antioxidant factors total antioxidant capacity (TAC), total antioxidant status (TOS), Malondialdehyde (MDA), Catalase (Cat) in different groups. Data analysis in different groups was done with SPSS software and one-way variance statistical test and  $P < 0.05$  was considered significant.

### RESULTS AND DISCUSSION

The results showed a decrease in TAC, Catalase and an increase in TOS, MDA in the group infected with *Escherichia coli* bacteria compared to the control group ( $P < 0.001$ ). Also, the change in the level of antioxidants in the group treated with probiotic mixture was significantly compared to the infected group.

### CONCLUSION

The present study showed that the protective effect of probiotic mixture on *Escherichia coli* infection of testicular tissue antioxidant factors may be related to the antioxidant function and free radical scavenging of proboscis.

**Keywords:** Probiotic, Antioxidant, *Escherichia coli*, testis, rat

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigation of *Xanthomonas* bacteria causing citrus canker

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#### ABSTRACT

The origin of citrus canker disease is from Southeast Asian countries. In general, the severity of bacterial canker disease is in areas that have rain in spring or the season of growth and development of branches. Citrus bacterial canker has different forms.

*Xanthomonas axonopodis* pv. *citri* (Xac) is the phytopathogen responsible for citrus canker, one of the most devastating citrus diseases in the world. A broad range of pathogens is recognized by plants through so-called pathogen-associated molecular patterns (PAMPs), which are highly conserved fragments of pathogenic molecules. In plant pathogenic bacteria, lipopolysaccharide (LPS) is considered a virulence factor and it is being recognized as a PAMP. The study of the participation of Xac LPS in citrus canker establishment could help to understand the molecular bases of this disease. In the present work we investigated the role of Xac LPS in bacterial virulence and in basal defense during the interaction with host and non-host plants. We analyzed physiological features of Xac mutants in LPS biosynthesis genes (*wzt* and *rfb303*) and the effect of these mutations on the interaction with orange and tobacco plants. Xac mutants showed an increased sensitivity to external stresses and differences in bacterial motilities, *in vivo* and *in vitro* adhesion and biofilm formation. Changes in the expression levels of the LPS biosynthesis genes were observed in a medium that mimics the plant environment. Xac $wzt$  exhibited reduced virulence in host plants compared to Xac wild-type and Xac $rfb303$ . However, both mutant strains produced a lower increase in the expression levels of host plant defense-related genes respect to the parental strain. In addition, Xac LPS mutants were not able to generate HR during the incompatible interaction with tobacco plants. Our findings indicate that the structural modifications of Xac LPS impinge on other physiological attributes and lead to a reduction in bacterial virulence. On the other hand, Xac LPS has a role in the activation of basal defense in host and non-host plants.

**Keywords:** *Xanthomonas*, citrus canker, *Xanthomonas axonopodis*, plant pathogens, leaf spot

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Taking a fresh perspective on the antibiotic resistance of *Helicobacter pylori* and exploring innovative remedies

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The presence of *Helicobacter pylori* can cause gastric and extra-gastric diseases, including cancer, brain and nerve diseases, arteriosclerosis, arthritis, blood and eye diseases, changes in drug metabolism, and disorders in disease treatment. Widespread antibiotic resistance and the lack of a successful vaccine used in clinics have yet to be addressed due to the powerful ability of *Helicobacter pylori* to evade host immune attacks. There is an urgent need to discover new targets and develop new drugs to fight *Helicobacter pylori*.

*Helicobacter pylori* and its extra-gastric effects caused by metabolites or OMVs (outer membrane vesicles) can be treated with new methods including probiotics and microbiome regulation, biotechnology methods, optogenetics (a new method that uses light and waves to control bacterial activity and gene expression), oxygen therapy, antimicrobial peptides, phage therapy, and changed lysines. A new study showed that sitafloxacin is a potentially ideal drug for *Helicobacter pylori*.

New treatment methods using blue light waves and wavelengths during endoscopy can be a suitable and minimally invasive method without affecting healthy cells.

The gram-negative bacterium discovered in 1983 by Barry J. Marshall and J. Robin Warren, the bacterium *Helicobacter pylori*, causes gastritis and ulcers by infecting the stomach epithelium. It may also cause cardiovascular and neurological disorders, as well as blood abnormalities. We need research to understand the mechanisms and develop prevention and treatment strategies, including new medicinal and herbal substances, biotechnology, and nano-biotechnology methods.

### MATERIALS AND METHODS

New scientific articles

### RESULTS AND DISCUSSION

*Helicobacter pylori* causes chronic gastritis. It was discovered in 1983 by Barry J. Marshall and J. Robin Warren. It affects the digestive system and other organs, through the metabolites and OMVs produces, through the blood and nerves. We need new treatment methods to inactivate the bacteria, its metabolites, and extracellular vesicles.

### CONCLUSION

H. Pylori infection is harmful to all organs due to its metabolites and requires new treatments.

**Keywords:** *Helicobacter pylori*, cardiovascular, extra-gastric diseases, optogenetics, Alzheimer's disease, chronic inflammation, atherosclerotic plaques,

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Promising role of probiotics in cancer prevention and treatment: An overview

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Probiotics are live bacteria that have positive effects on health. The human body naturally contains these beneficial bacteria, particularly in the digestive tract. According to recent studies, changes in the gut microbiota can influence how certain cancer types start and progress. Scientists are now looking at probiotics' possible function in preventing cancer as a result of this. Probiotics' function in supporting gut health and their potential to reduce the risk of cancer will be covered in this review. We discuss the most recent research and examine the probiotics that have been researched in connection to cancer.

Probiotics work in the treatment and prevention of cancer through various multifaceted methods. They involve making anti-cancer chemicals, enhancing the immune response, preventing the proliferation of cancer cells, and promoting the health and differentiation of healthy cells. According to research, increased natural killer cells, which are essential for destroying malignant cells, have been found to be stimulated by probiotics. They can also control inflammation and support a balanced gut flora, which can reduce the risk of chronic inflammation, which is a major factor in the development of cancer.

Lactobacillus and Bifidobacterium are two common probiotic species. Each species has several strains, and each species can offer specific health benefits. For example, Bifidobacterium longum and Lactobacillus acidophilus show promise in preventing the spread of colon cancer cells. These probiotics improve digestion, boost the immune system, and improve overall health by rebuilding and regulating gut bacteria. Because probiotics increase the growth of beneficial gut bacteria and strengthen the intestinal barrier, they can reduce the side effects of conventional cancer treatments such as radiation and chemotherapy and improve overall gut health during cancer treatment. Probiotics' possible role in cancer prevention has been the subject of several investigations. These investigations seek to determine the possible preventive mechanisms probiotics may use as well as the degree to which they might lower cancer risk. Early studies have produced positive results, but additional research is required to completely comprehend the mechanisms underpinning probiotics' capacity to prevent cancer.

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### MATERIALS AND METHODS

A thorough search using the terms "probiotics," "anticancer activity," "gut microbiota," "microbiome," and "inflammation" was performed in databases including Google Scholar, Medline, and PubMed. This study was developed after a thorough analysis of relevant English-language articles and review articles. First, articles on the gut microbiota and its relationship to cancer were evaluated, followed by articles on new research to develop effective treatments.

### RESULTS AND DISCUSSION

Probiotics are expected to provide a solution to the problems associated with cancer treatment and become an important component of cancer management and prevention in the near future. The properties of probiotics open the way for several useful applications in cancer prevention techniques. Probiotics can also reduce the side effects of chemotherapy and surgery when administered together. In addition, probiotics can make chemotherapy drugs more effective. Another successful and promising approach that uses probiotics is targeted drug delivery.

### CONCLUSION

The properties of probiotics make them effective in the treatment and prevention of cancer. As an innovative and safe approach to cancer treatment, food supplements can provide a new approach to reducing the incidence of cancer and improving the patient's quality of life. Although more research is still needed, probiotics can be helpful. Better reporting of clinical trial results and adverse events is needed to increase the accuracy and validity of findings in subsequent updates.

**Keywords: Probiotics, inflammation, intestinal microbiota, Anticancer activity**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Phylogenic grouping of *Escherichia coli* isolated from milk samples of bovine mastitis in the industrial farms of Hamedan County

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

Mastitis is an inflammation of the mammary gland tissue because of various agents, the most important cause of the mastitis is the entry of pathogenic microorganisms, especially bacteria into the breast tissue and then reproduction and production of toxins by them. This disease can cause changes in the quality and quantity of milk, and for this reason, it is considered as one of the most important diseases in dairy cow's industries. One of the most common pathogens associated with clinical mastitis in cattle is Mammary Pathogenic *Escherichia coli* (MPEC). The MPEC strains could be responsible for economic loss in the dairy cattle and has zoonotic importance as well. Several methods including serotyping, pathotyping and phylothping have been used for *E. coli* typing and assigning. The aim of the present study was to assignment of the phylogenetic group in MPEC isolated from milk sample of cows with clinical mastitis.

#### MATERIALS AND METHODS

The present study was carried out on MPEC strains ( $n= 65$ ) isolated from milk sample of cows with clinical mastitis in industrial dairies of Hamadan county. First, genomic DNA was extracted from bacterial strains using rapid boiling method. Extracted DNA was then assigned to phylogenetic typing using the revised Clermont *E. coli* phylotyping method, a Quadruplex PCR based procedure.

#### RESULTS AND DISCUSSION

The Clermont *E. coli* phylotyping method results revealed that the 65% of MPEC isolates can be assigned to a phylogroups (A, B1, B2, C, D, E, F and *Escherichia* clade I), and 35% of isolates were not ascribable to any group. Out of 65 MPEC strains the predominant phylogroup was phylogroup B1 (48 %) followed by E (11 %).

#### CONCLUSION

Current study findings indicate that most predominant phylogroup of MPEC isolated from milk sample of cows with clinical mastitis in industrial dairies of Hamedan county was B1 phylogroup. Additionally, Clermont *E. coli* phylotyping method recommended as a useful and inexpensive genetic tool for MPEC isolates typing. However, further studies and application of new methods such as, Whole-genome sequencing, MLST typing are suggested for complete MPEC isolates typing.

**Keywords:** Bovine mastitis, Dairy cattle, Phylotyping, Mammary Pathogenic *Escherichia coli* (MPEC), PCR, Zoonosis.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Vitamin K<sub>2</sub> screening in *Bacillus* spp. isolated from soil samples of Tehran, Golestan, Markazi and Mazandaran provinces

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#### ABSTRACT

Vitamin K<sub>2</sub> (menaquinone, MK-n) is one of the three types of vitamin K, which produced by bacteria (1, 2). It has an important role to prevents osteoporosis and cardiovascular disease (2). Insufficient intake in food sources has made researches valuable to achieve high vitamin K<sub>2</sub> producer strains (3, 4, 5). In present study, soil *Bacillus* populations screened for menaquinone production potency. Ten soil sample collected from different part of central and northern regions of Iran. In the investigation of 97 isolated strains for vitamin K<sub>2</sub> production through liquid state fermentation in specific media, the vitamin concentration measured in 248nm UV-spectroscopy following extraction by n-hexane: 2-propanol (6). Analysis approved through potent menaquinone producer isolates by thin layer chromatography (TLC) and menaquinone structure determined by liquid chromatography- mass spectrometry (LC-MS) in Superior isolates. Their chromatogram showed Menaquinone-7 and menaquinone-12 contents in JM9 and MG2 extracts and menaquinone-7 by PK13. They characterized by gram straining and 16SrRNA sequencing (7); respectively *Bacillus cereus* JM9, *Bacillus cereus* MG2 and *Bacillus albus* PK13 It was the first report of isolation *Bacillus cereus* and *Bacillus albus* producing vitamin K<sub>2</sub> from soil origin. Due to the key role of bacteria in the biological production of vitamin K<sub>2</sub>, superior isolates with natural capability are valuable. They can be optimized for higher quantities and applied as candidate for future biotechnological application.

**Keywords:** Menaquinone, Bacteria, Optical Density, Chromatography

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and Determination of Vitamin K<sub>2</sub> Producer Bacteria from Iranian Traditional Cheese Samples: *Lactococcus lactis* and *Leuconostoc mesenteroides*

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#### ABSTRACT

Vitamin K<sub>2</sub> (menaquinone) is a form of vitamin K produced by bacteria. Dietary use of this vitamin is limited with soybean consumption food products. Products containing menaquinone help take advantage of its valuable health benefits. Cheese is a dairy product with the contents of menaquinone. Nine traditional Iranian cheeses were tested for the presence of menaquinone-producing bacteria by optical density at 248nm and HPLC analysis since this vitamin content is thought to be linked to the cheese's bacterial makeup. Superior isolates characterization by 16S rRNA analyzed them *Lactococcus lactis subsp lactis* CHT8, and *Leuconostoc mesenteroides* CHG1. Regarding the presence of these two bacteria in cheese production at different stages, including a starter or as probiotics, adding the feature of menaquinone production will offer a precious cheese.

**Keywords:** Menaquinone, Reipening, starter, Food

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Bioconversion of hemicellulosic sugars into xanthan gum by isolated *Xanthomonas campestris* strain

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Xanthan gum, a widely utilized food additive known for its thickening, stabilizing, and emulsifying properties, is traditionally produced through fermentation using *Xanthomonas campestris* bacteria on glucose or sucrose-based media. However, there is increasing interest in investigating alternative feedstocks to enhance sustainability and reduce costs. This study examines the potential of isolated *Xanthomonas campestris* strains to convert hemicellulosic sugars from biomass into xanthan gum.

### MATERIALS AND METHODS

*Xanthomonas* sp. was isolated from walnut green shell samples in Marand City. Phenotypic tests were conducted on the isolates, including gram staining, biochemical reactions, and physiological assessments. Molecular identification involved DNA extraction using the phenol/chloroform method and confirmation through PCR amplification of the 16S rRNA gene. The resulting PCR products were sequenced and analyzed using the BLAST program in the NCBI database for specie identification. To prepare hydrolysate containing hemicellulosic sugars, sorghum stem (10% w/v) was stirred in acetone/water solution (50%) with 0.1% sulfuric acid. The mixture was pretreated at temperatures 120, 150, and 180 °C for 30 or 60 minutes. The liquid phase was used to produce hemicellulose xanthan gum.

### RESULTS AND DISCUSSION

This study investigated the production of hemicellulosic xanthan gum from sorghum stalk hydrolysate using organosolv pretreatment. The impact of temperature and duration on xanthan gum yield was examined, revealing a negative relationship between pretreatment intensity and yield. However, a maximum yield of approximately 12.78 g/L was achieved. Characterization studies indicated that the hemicellulosic xanthan gum had lower viscosity than standard xanthan gum. This approach enables the direct production of xanthan gum with reduced viscosity suitable for edible applications.

### CONCLUSION

To the best of our knowledge, this is the first report on the bioconversion of hemicellulose sugars into xanthan gum, offering valuable insights for future research and industrial applications.

**Keywords:** Xanthan gum, Hemicellulose, *Xanthomonas campestris*, Sorghum, Organosolv treatment.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### **Synthesis and characterization of the polyvinyl alcohol/starch/chitosan antibacterial film reinforced with NiO-CuO nanoparticles for food packaging**

**Fatemeh Momtaz, Elham Momtaz, Farkhondeh Poursina, Mahdieh Momtaz, Masoud Ayatollahi Mehrgardi, Tahmineh Narimani**

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#### **ABSTRACT**

Packaging is of great importance for maintaining the quality and preservation of food. Nowadays, antibacterial packaging for food gets very popular. In this study, nanocomposite films of polyvinyl alcohol/starch/chitosan (PVA/ST/CS) along with nickel oxide-copper oxide nanoparticles (NiO-CuONPs) are prepared for food packaging. For this purpose, NiO-CuONPs were synthesized by the co-precipitation method and structural characterization of nanoparticles (NPs) was carried out by XRD, FTIR and SEM techniques. Composites of PVA/ST/CS containing different percentages of NPs were prepared by casting method. The interaction between polymer compounds and NPs was confirmed by FTIR. Also, the distribution of particles within the composite was investigated by FESEM. The mechanical, diffusion barrier and thermal stability properties were determined. The results of mechanical tests showed that adding NPs up to 1% improves the mechanical properties (TS=31.94 MPa), while 2% of NPs decreases the TS to 14.76 MPa. The toxicity on fibroblast cells and the antibacterial activity of the films against Gram-positive and Gram-negative bacteria were investigated. As a result, the antibacterial properties of the films were improved and these films did not show any toxicity. All in all, the bio-nanocomposite films involving NiO-CuONPs can be a promising proposal for food packaging.

**Keywords: Polyvinyl alcohol, Starch, Chitosan, Nickel oxide-copper oxide nanoparticles, Food packaging**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular Analysis of Catheter-Associated *Proteus mirabilis* Isolates: Insights into Adhesion and Antibiotic Resistance

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Proteus mirabilis* is a prominent uropathogen responsible for catheter-associated urinary tract infections (CAUTIs). It colonizes the urinary tract and forms biofilms that confer protection against antibiotics. This study aimed to investigate the presence and expression of specific genes associated with adhesion and antibiotic resistance in catheter isolates, particularly focusing on *mrpA* and *pmfA* as adhesion genes.

### MATERIALS AND METHODS

A total of 385 non-duplicate catheters were collected from intensive care unit (ICU) patients across multiple hospitals in Isfahan. The catheter solutions were cultured on blood and MacConkey agar, followed by conventional biochemical tests. Additionally, PCR and multiplex PCR techniques were employed to identify resistance genes in the isolates. Real-time PCR was performed to analyze the expression levels of the two adhesion genes.

### RESULTS AND DISCUSSION

Among the isolated *P. mirabilis* bacteria, 35% (14/40) harbored the *K2* capsular gene, while none exhibited the *K1* gene. The *bla<sub>NDM</sub>* gene was not detected; however, 60% of the isolates tested positive for the *acc* gene. The frequencies of *bla<sub>OXA23</sub>*, *bla<sub>OXA24</sub>*, and *bla<sub>OXA58</sub>* were 10%, 2.5%, and 2.5%, respectively, with one isolate displaying positivity for all three genes. No instances of *bla<sub>SIM-1</sub>* and *bla<sub>VIM1</sub>* were observed in the isolates. Furthermore, no significant correlation was found between capsular genes and biofilm formation. Real-time PCR analysis revealed elevated expression levels of *mrpA* and *pmfA* genes in clinical isolates compared to the control strain obtained from urine.

### CONCLUSION

The presence of carbapenems genes (OXA-23, OXA-24, and OXA-58) in *P. mirabilis* isolates underscores the dissemination of resistance genes among different bacterial species and emphasizes the significance of infection control measures in healthcare settings. Moreover, the involvement of acetyl-CoA biotin decarboxylase (ACD) in metabolism, indole production, and biofilm formation highlights the intricate nature of *P. mirabilis* pathogenesis. Enhanced comprehension of the molecular mechanisms underlying these processes could pave the way for novel strategies aimed at prevention and treatment of *P. mirabilis* infections.

**Keywords:** *Proteus mirabilis*, catheter-associated urinary tract infections, Real-time PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating the quorum quenching effect of allicin by in silico method on *Pseudomonas aeruginosa*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The expression of most pathogenic factors in *Pseudomonas aeruginosa* bacterium is regulated by a signaling system called quorum sensing, which is used to interfere with this cellular communication by the quorum quenching system. In this research, the antimicrobial effect of allicin was investigated to prevent the spread of infection and inhibit the quorum sensing of *Pseudomonas aeruginosa* in the in-silico environment.

#### MATERIALS AND METHODS

In this study, information about the LasR protein structure of *Pseudomonas aeruginosa* was first obtained from the protein data bank website. Then, by using the molecular dynamics simulation method in the in-silico environment and with the help of various software in the field of bioinformatics such as Gromacs, the effect of allicin, which is chemically and three-dimensionally similar to the specific ligand of the LasR protein, named acyl homoserine lactone, to compete and inhibit the quorum sensing system of *Pseudomonas aeruginosa* in a period of 300 nanoseconds was performed.

#### RESULTS AND DISCUSSION

According to the findings of this research, the structure and function of allicin were similar to the structure of the LasR protein of *Pseudomonas aeruginosa*, which was obtained in silico environment through Auto Dock Vina software and molecular dynamics simulation of the connection between protein and ligand, which is probably allicin through competition and effect anti-quorum sensing exerts its antimicrobial effect.

#### CONCLUSION

The results showed that plant compounds such as garlic, which have high amounts of allicin, can be used as a natural compound with the anti-quorum sensing system of *Pseudomonas aeruginosa*.

**Keywords:** *Pseudomonas aeruginosa*, quorum sensing, quorum quenching, allicin, dynamic simulation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The effects of Antibiotics on cancer risk and treatment; outcomes and prospects: An overview

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Cancer is a common and frequently occurring disease that kills more than eight million people each year. Although a small percentage of cancer cases have a genetic component, it is important to note that cancer is an environmental disease, and the risk of developing cancer can be influenced by both extrinsic factors (such as carcinogens, pollutants, radiation) as well as intrinsic factors (such as metabolic, immune, or genetic deficiencies). In today's world, cancer can be treated with surgery, chemotherapy, radiotherapy, and immunotherapy in combination or individually. Various antibiotics have also been used to treat cancer, including Adriamycin, also known as doxorubicin and other anthracyclines produced by *Streptomyces* spp. Anticancer regimens include various antibiotics capable of alkylating DNA (e.g., Adriamycin, also known as doxorubicin, and others). Additionally, several cancer chemotherapy drugs (such as cisplatin, still used as a standard of care for human cancers such as testicular tumors) have antimicrobial properties. In addition, increasing studies have shown that antibiotics may disrupt the intestinal microbiota, causing chronic inflammation, altering normal tissue metabolism, causing genotoxicity, and weakening the immune system against bacterial malnutrition. As a result, cancer treatment can be adversely affected. As such, antibiotics are the type of compounds that may increase the risk of developing cancer, because they are capable of acting as extrinsic environmental carcinogenic factors (direct action) and altering the normal balance of the microbiota towards a more pro-carcinogenic composition (indirect action). Antibiotics' unquestionably advantageous effects on bacterial pathogens may therefore be overshadowed by the potential for concurrently raising the chance of developing cancer, which could lead to the emergence of secondary malignancies and the advancement of those cancers to advanced stages, including metastasis or tumor recurrence. The overuse of antibiotics by individuals through self-medication or sharing prescriptions, and the misguided indications and inappropriate dosing schemes prescribed by physicians and medical institutions for treating clinical cases that don't even involve bacterial infections, illustrate the importance of this issue. Additionally, patients are frequently

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exposed to antibiotics following surgery and when chemotherapeutic drugs are suppressing their immune system.

### MATERIALS AND METHODS

A comprehensive search was conducted on databases such as Google Scholar, Medline, and PubMed using the phrases "antibiotics," "microbiom," "cancertherapy," "microbiota," "tumor microbiota," "FMT," and "dysbiosis." An extensive evaluation of significant English-language papers and review articles served as the basis for the investigation.

### RESULTS AND DISCUSSION

Introducing new antibiotics that may have both antimicrobial and anti-tumor properties, bacteriophages, or enzybiotics, which may have comparable benefits in terms of their antimicrobial activity but do not produce collateral issues related to resistance, microbiota dysbiosis, or a reduction in the response to anti-tumor therapies, are alternatives to the current patterns of antibiotic use. Using oncology-specific microbiota modulation techniques to reduce dysbiosis. The use of probiotics, prebiotics, or symbiotic supplements or, more directly, fecal microbiota transplantation (FMT) are two clear main alternatives to microbiota alteration procedures.

### CONCLUSION

Alternatives to antibiotic use that either don't induce or very mildly cause microbial dysbiosis offer potentially helpful tactics to control the carcinogenic process. The human tumor microbiome is an additional aspect that should be taken into account. Technology advancements have only recently made it possible to distinguish tumor microbiome signals from those obtained from genetic investigations of the normal microbiota. However, due to the very low bacterial biomass present in tumors, detailed characterization of the tumor microbiota has not advanced at a rapid rate. Despite this situation, an exciting finding has recently been reported demonstrating that different tumor types have distinct microbiome signatures, which has important implications from a diagnostic point of view, and Moreover, it is significant that intracellular bacteria make up the tumor microbiome. Application of system biology approaches will be necessary to comprehend the role of intracellular bacteria specific to a given tumor type in maintaining the balance of the normal microbiota and the effects of antibiotics on cancer risk and treatment effectiveness.

**Keywords: Antibiotics, cancer risk, dysbiosis, Cancer therapy, Microbiome**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Designing a biofilter odor removal system for a sewage lifting station

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Biofiltration is an air pollution control technology that uses microorganisms to biodegrade odors and other volatile pollutants in waste air streams. Microorganisms exist on the surface and in the thin layer of water that surrounds the surface of the biofilter material. During the biofiltration process, polluted air slowly passes through the biofilter bed. Pollutants are absorbed on the surface of the substrate and penetrate into the water layer. Simultaneously, microorganisms consume it and produce energy, biomass and metabolic end products, mainly CO<sub>2</sub> and H<sub>2</sub>O. Biological air filtration is performed in special bioreactors, i.e., biofilters (BF), bio-trickling filter (BTF), bio-scrubbers (BS), which can remove a wide range of gaseous pollutants present in low concentrations in large amounts of air.

### MATERIALS AND METHODS

In this study, a biofilter system was designed to remove emitted odors (H<sub>2</sub>S) from a sewage lifting station with a flow rate of 650 m<sup>3</sup>/h. This system includes 2 tanks each with a capacity of 10 m<sup>3</sup>. The loading rate was 100 m<sup>2</sup>/m<sup>3</sup>.h and the hydraulic retention time was 83 seconds. The system is equipped with lignocellulosic media surface moistening system.

### RESULTS AND DISCUSSION

The biofilter reactor inoculated with *Thiobacillus spp.* microbial fertilizer, activated sludge, working biofilter effluent and chicken manure are used. To increase the growth of sulfur-oxidizing microorganisms and biofilm formation, water containing mineral nutrients was used for several weeks. Under stable operating conditions, the concentration of H<sub>2</sub>S was reduced from 16 mg/L to less than 1 mg/L (more than 93% removal).

### CONCLUSION

Today, biofiltration is considered a half-century old technology that emerged in industry and is used in waste gas management and odour removal. In general, the presence of any type of chemical pollutant, even if their concentration is variable, biofiltration can guarantee the removal of more than 50% of it. The lifetime of lignocellulosic media in biofilters is usually up to 5 years.

**Keywords:** Biofiltration, Odor removal, Sewage lifting station, *Thiobacillus spp.*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the prevalence of beta-lactamase CTX-M resistance gene, its polymorphism and sequencing in *Escherichia coli* bacteria isolated from urinary infection samples collected from Tabriz hospitals.

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

One of the most common bacteria in the Enterobacteriaceae family is *Escherichia coli*, which causes urinary tract infections (UTI). UTI is one of the most common bacterial infections. Since *E.coli* is constantly exposed to antibiotics, antibiotic resistance has increased in this bacterium. From two decades ago, *E.coli* isolates producing CTX-M have been reported in the community and hospital environments from all over the world. The aim of this study was to determine the prevalence of bla CTX-M resistance gene and its subtypes in *E.coli* clinical isolates in Tabriz city.

### MATERIALS AND METHODS

In this cross-sectional study, 200 clinical samples were collected and verified from the educational hospitals of Tabriz during a period of 6 months from April 2019 to October 2019. Antibiotic sensitivity test was done by disk diffusion method and the confirmatory test was done by combined disk method to identify ESBL producing strains. DNA of ESBL producing isolates was extracted and the PCR test was performed for the CTX-M gene. Using the RFLP method, CTX-M groups were identified and 10 isolates were randomly selected and sequenced.

### RESULTS AND DISCUSSION

In this study, 90 isolates (45%) were ESBL producing isolates. Among the positive phenotype isolates, 92.22% had the CTX-M gene, of which 85.18% were CTX-M<sub>1</sub>. The sequenced isolates were more than 96% identical to CTX-M<sub>15</sub>.

### CONCLUSION

The prevalence rate of CTX-M in Tabriz is high and the predominant group is CTX-M<sub>1</sub>, which requires quick and appropriate measures to prevent it.

**Keywords:** *Escherichia coli*, UTI, ESBL, CTX-M, Tabriz

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Comparison of growth rate and protein content by *Spirulina Platensis* in Photobioreactor and Shake Flask

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Spirulina* is known for its high nutritional value and potential industrial applications. The influence of cultivation conditions on the growth of the cyanobacterium *Spirulina platensis* was investigated by using two types of methods, bubble column photobioreactors, and shaker Erlenmeyer flasks, to compare growth performance, biomass productivity, and protein content.

### MATERIALS AND METHODS

Cultures were grown in Zarrouk's medium and exposed to a range of light intensities (3000 LUX), with a light/dark cycle of 16/8 h. at room temperature, pH 9.5, Providing aeration for the photobioreactor with a central bubble column in which the intensity of aeration and mixing was set at 1000 ml/min 8-hour intermittent aeration during 24 hours. and flask aeration was provided by the digital shaker which was adjusted for 2 hours stirring and 2 hours resting continually to provide sufficient aeration and mixing. bubble column photobioreactors and Erlenmeyer flask compared for their performances during the cultivation of *Spirulina platensis*. Culture conditions were kept the same and different parameters were examined through the experiments.

### RESULTS AND DISCUSSION

The results showed that the photobioreactor yielded significantly higher biomass concentration and growth rate. After 12 days, the bioreactor achieved 3.27 g/L of biomass while the flasks only reached 2.12 g/L, Subsequently, on the 5th day, the growth in the photobioreactor was 10% higher than the flask and continued to the 8th day, after that this difference decreased. In Protein analysis the highest amount of protein in both environments was estimated on the 10th day of cultivation in terms of biochemical composition, the photobioreactors produced *Spirulina* with higher protein (55-60% vs 40-45%) levels compared to the shake flasks.

### CONCLUSION

The higher yields in the photobioreactors are attributed to better light penetration and distribution, as well as improved mixing and mass transfer. In the shake flasks, much of the light is absorbed or reflected in the liquid-air interface rather than penetrating the culture. Ultimately, the choice between a photobioreactor and an Erlenmeyer flask depends on the specific requirements and goals of the *Spirulina* cultivation.

**Keywords:** Photobioreactors, Growth rate, *Spirulina*, Erlenmeyer flask, Protein content.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating the level of aflatoxin M1 in Sarshir by ELISA in Karaj city

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Dairy products are mainly susceptible to contamination with aflatoxin M1 (AFM1), which can cause harmful effects such as hepatotoxicity, mutagenicity, toxigenicity, teratogenicity, carcinogenicity, neurotoxicity and cell toxicity, as well as suppressing the immune system and neoplasticity. Considering the people's acceptance of the consumption of dairy products such as Sarshir, as well as the significant risks of AFM1 in consumers, the investigation of contamination with this substance in traditional Sarshir dairy products was carried out in different areas of Karaj city.

#### MATERIALS AND METHODS

40 samples of Sarshir products from traditional yogurt shops from different parts of Karaj city (northwest, northeast, southeast and southwest) from each region, 10 samples were purchased in sterile containers, 100 grams each. The samples were transferred to the laboratory at a temperature of 4 degrees Celsius and up to 24 hours, and then they were evaluated for aflatoxin M1 contamination using the ELISA method

#### RESULTS AND DISCUSSION

The obtained results indicate that aflatoxin M1 in Sarshir samples in different areas of Karaj city is significantly lower than the permissible limit (100ppt). The lowest value was 12 ppt and the highest value was 32 ppt. (Mean =  $14.70 \pm 3.49$  ppt). The no significant difference is observed between amount of aflatoxin in Sarshir samples from the defferent places of Karaj ( $p > 0.05$ ).

#### CONCLUSION

According to the results obtained from this study, the amount of aflatoxin in Sarshir samples collected from Karaj city was lower than the permissible limit. Monitoring the way this product is prepared and distributed, as well as checking the amount of aflatoxin in the final product, can be effective in preventing possible contamination. The way to prepare Sarshir and the steps used in it (including the selection of raw milk, the containers used, the amount of boiling, its chemical and microbial characteristics) can be a reason for reducing the amount of aflatoxin in this product.

**Keywords:** Aflatoxin, Sarshir, Karaj City, Traditional Dairy Product

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Throat culture in children with cystic fibrosis

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

The dangerous and fatal genetic disease called Cystic Fibrosis (CF) is caused by loss-of-function mutations in the gene encoding for the CFTR chloride/bicarbonate channel. The objective of this study was to investigate the swabs of throat cultures for children with cystic fibrosis.

##### MATERIALS AND METHODS

Throat swabs were collected from the *Mahdieh* Medical Laboratory in Isfahan, Iran. Throat swabs are an important part of disease surveillance for children who are unable to produce sputum. A total of 50 patients were enrolled in this study. The outcome of current research was approved by the *Mahdieh* Medical Laboratory Ethics Committee. To meet the purpose, all children with CF aged 4–12 years were targeted from April to July 2023.

##### RESULTS AND DISCUSSION

Four different organisms were isolated: *Pseudomonas aeruginosa* (44.5%), *Staphylococcus aureus* (Methicillin-resistant) (17%), *Streptococcus Pyogenes* (22%), and *Candida* spp. (16.5%). *S. aureus* (MRSA) was found to be sensitive to Ciprofloxacin and Gentamycine. Meanwhile, Fungal isolates disclosed great sensitivity to Amphotericin B.

##### CONCLUSION

In conclusion, *Pseudomonas aeruginosa* was the most frequently isolated organism, which was largely sensitive to Ceftazidime and Meropenem. To treat *Streptococcus pyogenes*, the use of Penicillin is recommended for 10 days.

**Keywords:** Cystic fibrosis, *Pseudomonas*, *staphylococcus*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The antibacterial effect of an environmentally-isolated specific bacteriophage belonging to Myoviridae family in combination to polymyxin B against multidrug-resistant *Acinetobacter baumannii* clinical isolates

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The multidrug-resistant *Acinetobacter baumannii* (*A. baumannii*) has re-emerged as one of the predominant causes of hospital infections causing failure in infection eradication. Phage therapy has recently received attention, particularly against multidrug-resistant (MDR) infections. The aim of this study was assessment of the effect of specific bacteriophages against MDR-*A. baumannii* clinical isolates.

### MATERIALS AND METHODS

In this study, a specific bacteriophage was isolated from environment and identified morphologically using transmission electron microscopy (TEM). Additionally, MDR-*A. baumannii* were isolated and their susceptibility to single bacteriophage and in combination with polymyxin B was investigated.

### RESULTS AND DISCUSSION

Isolated bacteriophage (Ap38) had a polyhedral head ( $10 \pm 85$  nm) and short tail ( $50 \pm 5$  nm), belonging to *Myoviridae* family. The bacteriophage contained a collar at the end of the head. The simultaneous use of phage and polymyxin B had significantly higher antibacterial activity than single phage against *A. baumannii*.

### CONCLUSION

Therefore, the use of phage therapy in combination to antibiotics can be a promising approach for the elimination of drug-resistant infections.

**Keywords:** Bacteriophage, Phage therapy, Multidrug resistance, *Acinetobacter baumannii*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluation of bacteriophage efficacy in inhibiting methicillin-resistant *Staphylococcus aureus* isolated from patients with external ear infection in Vali-e-Asr Hospital in Fasa

Javad Jokar

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

Otitis externa is a common inflammation in the external ear canal and eardrum. Otitis externa can be acute or chronic, with the acute form affecting four out of 1,000 people annually and the chronic form affecting 3-5% of the population. If left untreated, the acute disease can be followed by edema, discharge, pain, and finally, extra-canal manifestations. Due to the increase in the use of antibiotics in society, it is necessary to conduct various studies to control these bacterial agents in medical centers.

##### MATERIALS AND METHODS

In this study, methicillin-resistant *Staphylococcus aureus* was isolated from patients with external ear infections in Fasa Wali Asr Hospital, its specific bacteriophages were isolated from the environment, and its antimicrobial properties were investigated.

##### RESULTS AND DISCUSSION

The results showed that the bacteriophages significantly reduces the number of bacterial colonies.

##### CONCLUSION

The obtained results showed that bacteriophages act specifically. Considering that the level of resistance to antibiotics is increasing, bacteriophages can be a suitable alternative to the use of antibiotics in the treatment of bacterial infections.

**Keywords: Phage, Phage Therapy, Otitis Externa, Antibiotic Resistance, Methicillin-Resistant *Staphylococcus Aureus*.**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Co-immobilization of Carbohydases and Proteases by Nanomagnetic Combi-CLEAs Method for Oil and Protein Hydrolysates Extraction from Oil Seeds in Aqueous Phase at Single Step Process

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Functionalized magnetic nanoparticles are effective enzyme carriers since they have several advantages such as easily separation under external magnetic fields as well as enhancing mass transfer and reusability of enzymes. In this article, three types of enzymes included Viscozyme L (carbohydases), Alcalase 2.4 L (endo-peptidase), and Flavourzyme (Exo-peptidase) Simultaneously were immobilized by nanomagnetic cross-linked enzyme aggregates (CLEAs) method. Then assessment of the results obtained was performed. At first, Fe<sub>3</sub>O<sub>4</sub> nanoparticles were synthesized by chemical co-precipitation then, surface coating procedure was used for functionalization of Fe<sub>3</sub>O<sub>4</sub> by lysine amino acid. Thereafter, enzyme aggregation and cross-linking was achieved by using of enzymes with functionalized Fe<sub>3</sub>O<sub>4</sub> and glutaraldehyde in saturated ammonium sulfate, and/or solvents such as, acetone, acetonitrile, tert-butanol, isopropanol, and ethanol at 3-4° C separately. Afterthat, for size reduction of formed NM-Combi-CLEAs, high speed homogenizer and then ultrasonic waves were applied. Then, produced NM-Combi-CLEAs was hold at 3-4° C for 3-24 hours and cross-linked enzymes were separated from liquid phase by centrifuge at 15000 rpm accurately. Finally, activity assay, FE-SEM images and EDX, DLS and zeta potential analysis, FTIR, degree of hydrolysis (DH%), kinetic parameters ( $K_m$ ,  $V_{max}$ ,  $k_{cat}$ ,  $k_{cat}/K_m$ ,  $t_{1/2}$ ,  $k_d$ ) and thermodynamic parameters ( $\Delta G$ ,  $\Delta H$ ,  $\Delta S$ ,  $E_{a(in)}$ ) of immobilized enzymes compared to native enzymes mixtures was evaluated. The NM-Combi-CLEAs kept 75-80% of its original activity after 10 cycles, which proposes strong operational stability. In conclusion, the NM-Combi-CLEAs are thermo-stable, reusable, and efficient nanobiocatalyst for enzymatic oil and protein hydrolysates extraction in aqueous phase.

In this present work, a new nanobiocatalyst is fabricated by using of co-immobilization of various enzymes (Viscozyme, Alcalase, Flavourzyme) in nano scale. This method consists of the simultaneous nano aggregation of the free enzymes in an appropriate solvent and cross-linking of aggregates containing functionalized Fe<sub>3</sub>O<sub>4</sub> by glutaraldehyde via  $\epsilon$ -NH<sub>2</sub> groups of accessible surface lysine residues.

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The produced efficient nanobiocatalysts displayed improvement in thermal stability and reusability. Moreover, mass transfer limitation, missed fine CLEAs during recovery processes are eliminated.

### MATERIALS AND METHODS

Viscozyme L produced from a selected strain of *Aspergillus aculeatus*, Alcalase 2.4 L, (from *Bacillus licheniformis*) and Flavourzyme 1000 L (from *Aspergillus oryzae*) were supplied by Novozymes (Bagsvaerd, Denmark). starch, pectin, casein, galacturonic acid, glutaraldehyde (25% v/v in water), sodium potassium tartrate, 3,5 dinitrosalicylic acid, FeCl<sub>2</sub>, FeCl<sub>3</sub>, D(+) glucose, L-lysine monohydrochloride (>99%), and ascorbic acid were purchased from Merck. Bovine serum albumin (BSA) was obtained from Fluka company. Coomassie brilliant blue (G-250) was purchased from GE Healthcare (Uppsala, Sweden). All other chemicals were supplied by Merck (Darmstadt, Germany).

### RESULTS AND DISCUSSION

In the present research, multi-enzyme immobilization in aqueous phase process in a single step process was used for oil and protein hydrolysates extraction from sesame seeds and rice bran. At first, the enzyme activity, protein content, and optimum process conditions for Viscozyme, Flavourzyme, and Alcalase, were evaluated. Then, the effect of coinciding use of proteases on the activity of Viscozyme was assessed. The results revealed that, 2:1 ratio of water/oil seeds, Viscozyme L amount 1.5 kg per ton of oil seeds, 0.1% Alcalase, 0.5% Flavourzyme per oil seeds protein at 55 ° C, pH 5.5, 6 h reaction time, and inactivation of enzymes at 85-80 ° C for 15 minutes were the optimal process conditions. The oil extraction efficiency was 93% with 83-84% oleic and linoleic essential fatty acids. The protein hydrolysates efficiency was about 3 times that of the original protein with molecular mass of 10-20 kDa and its methionine was raised about 2 times. The essential amino acid and nutritional indices were enhanced 51.34%, and 51.41% respectively compared to raw protein.

### CONCLUSION

The removal of toxic hexane solvent, using the simultaneously three types of enzymes in the aqueous phase process for oil and protein hydrolysates extraction with high efficiency is the novelty of this research. Thus, this process introduces as an efficient, eco-friendly and safe method.

**Keywords:** Enzyme immobilization, Nanomagnetite, Cross-linked enzyme aggregates, nanobiocatalyst, Kinetics and Thermodynamics.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The Study of the Genes Causing Resistance to Aminoglycosides in *Escherichia coli* Strains Isolated from Clinical Specimens

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### ABSTRACT

*Escherichia coli* is the most common cause of urinary tract infection (UTI), with a prevalence of 80%. Enzymatic inactivation of antibiotics of the aminoglycoside family by aminoglycoside-modifying enzymes is the primary mechanism of *E. coli* resistance to these pharmaceutical drugs, and the recent expression of the Methylase 16SrRNA gene in Gram-negative bacteria that cause high levels of resistance to these antibiotic medications are considered to be a significant concern. This study aimed to determine the antibiotic resistance pattern and the prevalence of antibiotic resistance genes against aminoglycoside antibiotics among clinical isolates of *E. coli* by PCR.

About 500 clinical *E. coli* isolates were recovered in 2017 from the hospitals of Ilam. Antibiotic susceptibility testing was determined against selected antibiotics using the disk diffusion method and E-test according to CLSI standards. The prevalence of antibiotic-resistance genes among clinical *E. coli* isolates was determined using PCR. Data were analyzed using SPSS software and the Chi-square test. A p-value less than 0.05 is considered statistically significant.

E-Test results showed that 72.4% and 71.3% of isolates were resistant to Tobramycin and Gentamicin, respectively. Also, 75(79.8%) and 91(96.8%) of *E. coli* isolates contained aac(3)-IIa, and aac.(6)-Ib, but other used genes were not detected in any isolate. Due to Gentamicin's increased use and availability, its resistance was higher than other antibiotics in many areas, such as Ilam. However, the frequency of resistance to these antibiotics generally varies from region to region, implying the need for antimicrobial susceptibility testing before treatment.

**Keywords:** *Escherichia coli*, Aminoglycoside-modifying enzymes, Gene, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Cure of *h.pylori* with probiotics

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Helicobacter pylori* is a bacterium that can cause gastrointestinal disorders such as peptic ulcer and gastric cancer. Antibiotics are the primary treatment for *H. pylori* infection, but the increasing resistance to antibiotics has led to the investigation of alternative treatments such as probiotics. Probiotics are live microorganisms. The most commonly used probiotic bacteria are *Lactobacillus* and *Bifidobacterium*. The main objective of using probiotics in the treatment of *H. pylori* infection is to improve the eradication rate of the bacterium and reduce the side effects of antibiotics.

#### MATERIALS AND METHODS

Obtaining probiotics from different types of bacteria requires different methods and materials. Encapsulation techniques have attracted great interest because they encapsulate a material in a polymeric membrane matrix without affecting its biological activity. We can use probiotics alone or in addition to conventional therapy. The effect of probiotics on *H. pylori* gastritis is commonly measured by rapid urease testing, respiratory urea testing (UBT), serological testing, fecal antigen testing, and histological examination of gastric biopsies.

#### RESULTS AND DISCUSSION

Probiotics have been shown to improve *H. pylori* eradication rates and reduce side effects during treatment. Benefits of using probiotics as an adjunct treatment for *H. pylori* include: Increased eradication rate of *H. pylori* infection. Reducing the side effects of antibiotics during treatment. Restoration of gastric dysbiosis caused by eradication therapy. In a few preliminary studies, oral administrations of *L. salivarius* to *H. pylori*-infected gnotobiotic BALB/c mice showed a highly protective and therapeutic effect. Similarly, Coconnier et al<sup>29</sup> reported that

#### CONCLUSION

Research has shown that some probiotic strains may help reduce *H. pylori* colonization and relieve symptoms. More research is needed to determine optimal strains, dosages, and treatment durations. Also, probiotics should not be used as the sole treatment for *H. pylori*, but rather as an adjuvant therapy to standard antibiotic therapy. Overall, although probiotics may play a role in the treatment of *H. pylori* infection, more research is needed to determine their effectiveness as cures.

**Keywords:** Probiotics, Helico bacter, Eradication

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Molecular Evaluation of Brucella infection in raw milk from the livestock of Famenin city (Famenin Brucellosis cohort study)

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

Brucellosis is one of the most important and well-known zoonosis in the world and especially in Iran. A timely and accurate diagnosis of this disease is the starting point of any effective program to control it in humans and animals. To detect Brucella bacteria in milk and dairy products, the rapid agglutination method and milk ring test with Brucella abortus antigen are one of the most important primary screening methods for the presence of Brucella bacteria. Also, the new diagnostic method that has high sensitivity and minimizes the risk of transmission of infection is polymerase chain reaction (PCR). In this study, we aimed to compare diagnostic methods for evaluating raw milk samples isolated from domestic animals in Famenin city of Hamedan province.

##### MATERIALS AND METHODS

In this study, 738 raw milk samples from bovine, sheep, and goats in the Famenin region were investigated. At first, the primary screening of the presence of Brucella bacteria in milk samples was carried out through the milk ring test using B. abortus antigen. Then, all seropositive samples were selected for molecular evaluation. The DNA from milk samples was extracted and utilized in the PCR technique using the BCSP31 target gene and IS711 locus.

##### RESULTS AND DISCUSSION

Out of 738 collected samples, 46 samples were positive in MRT (milk ring test). Among 46 seropositive samples, 42 (91.3%) samples were related to sheep, 4 (8.7%) samples to goats, and no bovine samples had positive results in MRT. 36/46 (78.26%) samples with positive MRT results were confirmed as Brucella genus using the BCSP31-PCR. Among the 36 confirmed samples, 6/36 (16.66%) samples as B. abortus and 30/36(83.33%) samples as B. melitensis were identified using IS711-PCR.

##### CONCLUSION

Our results of the primary serological examination of milk samples of animals did not show complete agreement with molecular examinations. This study showed that the PCR method has minimal biological contamination and high sensitivity and accuracy, especially in determining Brucella species.

**Keywords:** Brucella abortus, Brucella melitensis, livestock, milk ring test, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Microbial decolorization of food industry azo dyes isolated from industrial wastewater

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Azo dyes used in textile, pharmaceutical, and food industries have emerged as a major environmental hazard due to their toxic and carcinogenic properties. Conventional wastewater treatment methods are often insufficient in effectively removing these recalcitrant compounds, leading to their discharge into water bodies, resulting in severe ecological and health issues. As a promising alternative, microbial degradation has gained significant attention as an eco-friendly approach for the efficient removal of azo dyes from contaminated sewage. Isolation and investigation of microorganisms which are able to remediate food industry azo dyes were the aims of the current study.

### MATERIALS AND METHODS

To isolate the microbial consortium able to decolorize azo dyes, samples were collected from the activated sludge in a food industrial wastewater treatment system. The edible azo dyes consisted of tartrazine, sunset yellow, and carmosin. The collected samples were inoculated into 100 ml of R2A medium containing 50 mg L<sup>-1</sup> of individual dyes, and were then incubated at 28 °C for 7 days. Microbial decolorization was monitored using a spectrophotometer.

### RESULTS AND DISCUSSION

Results demonstrated that the microbial consortium were able to decolorize the dyes for approximately 60% after 24 hours. The sunset yellow and carmosin dyes were decolorized more than 90% after 48 and 72 hours, respectively. Furthermore, tartrazine was decolorized more than 40% after 72-96 hours. These results revealed that the microbial consortium was able to decolorize food azo dyes and can be applied in industrial wastewater treatment plants.

### CONCLUSION

These results revealed that the use of a microbial consortium with the capacity to decolorize azo dyes is an encouraging approach towards sustainable wastewater treatment system. This microbial consortium exhibits promising capabilities to decolorize azo dyes. These findings enhance our understanding of microbial decolorization processes and offer new prospects for addressing the growing concerns of azo dye pollution.

**Keywords:** Microbial decolorization, food azo dyes, industrial wastewater

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Assessment of antibacterial, antioxidant and cytotoxic potential of green synthesis of silver nanoparticles by extracts of *Allium akaka* S.G. Gmelin

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#### ABSTRACT

Applications of nanotechnology in different areas of research have expanded over the last years. Silver nanoparticles (Ag-NPs) have beneficial effects as antioxidants, antibacterial, and anticancer [1-2]. Valak is a native vegetable of Iran that includes several species of *melanocrommyum* subgenus belong to the *Allium* genus. Their fresh leaves are consumed as vegetable. Their fresh and onions are used in pilaf. The aims of the present work were to study the possible green synthesis of Ag-NPs using *Allium akaka* S.G. Gmelin aqueous leaves extract as a reducing agent; to characterize them, to investigate the antibacterial, antioxidant potency and cytotoxic potential of these biosynthesized Ag-NPs. The obtained nanoparticles were characterized using UV-Vis, FT-IR, XRD, and TEM. The antioxidant potential was evaluated by 2,2-- diphenyl-1-picrylhydrazyl (DPPH<sup>0</sup>) free radical scavenging assay. Disc diffusion method was used to evaluate the antibacterial activity of Ag-NPs against *Escherichia coli* and *Staphylococcus aureus*. Cytotoxic activity of biosynthesized Ag-NPs was tested against human cervical carcinoma cell line (HeLa). Results of characterization showed that Ag-NPs were regular spherical in shape, with average diameter of 40 nm. The green synthesized Ag-NPs exhibited good antioxidant and antibacterial potential. Cytotoxicity test revealed that green synthesized Ag-NPs had inhibitory activity against cancer cell line (HeLa) which was concentration dependent. Accordingly, the treatment of (HeLa) cancer cell line cells according to over 24 hours revealed that the cytotoxicity of the aqueous extract and synthesized nanoparticles are dose-dependent, with the IC<sub>50</sub> value was equal to 52.32 and 51.76 µg/ml respectively. In fact, Plant crude extract contains novel secondary metabolites such as phenolic acid, flavonoids, alkaloids and terpenoids in which these compounds are mainly responsible for the reduction of ionic into metallic nanoparticles formation. Ag-NPs were successfully synthesized from *Allium akaka* S.G. Gmelin leaves extract, which acts as reducing, capping, and stabilizing agents in the process. The synthesis procedure was simple, low cost, and eco-friendly. Finally, green synthesis of silver nanoparticles proved to be an ideal potential candidate for the medical application where antibacterial and anticancer activity is essential.

**Keywords:** *Allium akaka* S.G. Gmelin, Silver nanoparticles, HeLa cancer cell line, *Escherichia coli*, Antioxidant activity.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation, Identification and Optimization of the Growth Conditions for Lipase Production by *Bacillus* sp.

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Enzymes, which have important metabolic functions for cellular structures, have entered daily and economic life to be used for various purposes. Lipases are enzymes that break down the glycerol esters of fatty acids. They are a physiologically and commercially important group of enzymes as their use increases rapidly and steadily for various biotechnological applications. The objective of this study was to isolation, identification and optimization of the growth conditions for lipase production by soil *Bacillus* sp.

### MATERIALS AND METHODS

The isolation of bacteria producing lipase from soil was conducted using a TBA medium. The identification of bacterial isolates was achieved through a combination of morphological, biochemical, and 16S rRNA methods. Enzyme activity was assessed by titrimetric analysis. Then, nutritional and physical parameters were optimized for production of lipase by *Bacillus* sp. Also, effect of carbon sources (coconut oil, corn oil, glucose, maltose, sucrose, starch, soybean oil, sunflower oil, and olive oil), organic and inorganic nitrogen sources (yeast extract, corn step liquor, peptone, tryptone, ammonium phosphate, ammonium nitrate and ammonium sulfate), different metal ions (MnSO<sub>4</sub>, FeSO<sub>4</sub>, LiSO<sub>4</sub>, BaCl<sub>2</sub>, KCl, NaCl, CaCl<sub>2</sub>, CuSO<sub>4</sub>), different temperatures (35-60 °C), pH ranges (4-9), inoculum amounts (5-10%) on enzyme production were studied.

### RESULTS AND DISCUSSION

Out of the 56 enzyme-producing isolates screened on TBA medium, 7 was identified as excellent lipase producer. Among these, a bacterium that produced the enzyme with high activity were selected for the next stage. Analysis of the 16S rRNA sequences of these isolates revealed a remarkable 99% similarity with *Bacillus cereus*. Results showed that sucrose and ammonium phosphate are the optimal sources of carbon and nitrogen, respectively. CaCl<sub>2</sub> was found to be the best metal source. Optimal lipase activity was achieved at a temperature of 45°C, a pH of 7 and following a 7% of inoculum amounts.

### CONCLUSION

The current research explored our local environments, searching for bacterial isolates that produce lipase. It suggested that *Bacillus cereus* isolated from the soil had the potentiality for lipase production and can be used in industry.

**Keywords:** Lipase, *Bacillus cereus*, Growth conditions, Soil

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Non-Photosynthetic Roles of Cyanobacterial Phytochromes in *Spirulina* (*Arthrospira platensis*), as Affected by Different Light Colors

Aynaz Gharajeh<sup>1</sup>, Aryan Sateei<sup>2\*</sup>, Shadman Shokravi<sup>3</sup> Mehdi Ebadi<sup>4</sup>

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nowadays, spirulina is one of the most important cyanobacteria that is used as a food-drug supplement. The effect of light color on the growth of spirulina has also been studied in some previous studies. Also, the medicinal and useful properties of phycobilins as cyanobacterial phytochromes have been reported previously. On the other hand, unlike plant phytochromes, phycobilins are better known for their photosynthetic roles in phycobilisomes. In this research, the effects of light color on growth, pigment content and especially the non-photosynthetic roles of phytochromes in spirulina have been studied.

### MATERIALS AND METHODS

Cultivation of *Spirulina* (*Arthrospira platensis*) in glass containers with a volume of 500 ml, without aeration and under continuous light (1500 lux/m<sup>2</sup>) for 45 days at 22 to 25 degrees Celsius was carried out in a randomized complete block design in 4 replications. The light colors were provided by LED lamps and included white as control, yellow, red, green and blue color. The measured traits included the rate of growth and fresh weight, the content of non-phytochrome pigments, including chlorophyll a, total carotenoids, as well as the amounts of phytochromes including phycocyanin, allophycocyanin and phycoerythrin on one hand and the ratio between pigments on the other hand.

### RESULTS AND DISCUSSION

Based on the results, the effect of light color on growth was significant and the highest growth was obtained in white and yellow lights and the lowest was in green and blue lights. The growth in red light was more than the growth in green and blue lights and less than the growth in white and yellow lights. Also, the effect of light color on the amounts of chlorophyll a, total carotenoids, phycocyanin and allophycocyanin was not significant. However, the total amount of total phycobilins in white and yellow lights was significantly lower than other lights. Contrary to expectation, the effect of red light on the content of the red pigment, phycoerythrin, was positive and caused the greatest increase, significantly. Comparison of the ratio of pigments also showed that, only the effect of light color on the ratio of Phycocyanin to allophycocyanin was significant, and the highest amount was also obtained in red light. Also the correlation between the growth and phycoerythrin/phycocyanin or phycoerythrin/allophycocyanin ratio was significantly positive, but the growth and phycocyanin/allophycocyanin ratio were negatively correlated.

### CONCLUSION

The findings of the present work, clearly show that the gene orchestra regulating the growth changes of spirulina is more influenced by the phytochromic ratios rather than absolute amounts of pigments. This cyanobacterial phytochromic role is also comparable to phytochromic roles in the higher plants on the other hand, the significant increase in the total content of phycobilins in the red light, without severe decrease in the growth, and due to the antioxidant properties of phycobilins, may have practical value in optimizing the potential of spirulina as a medicinal-food supplement. It also seems that yellow light among visible lights is an important factor in maintaining the balance between phytochromic ratios and preventing disturbances leading to the reduction of spirulina growth.

**Keywords:** Allophycocyanin, Blue-green algae, Carotenoid, Chlorophyll, Microalgae, Phycobilin, Phycocyanin, Phycoerythrin, Red light, Yellow light

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Phenotypic and Genotypic investigation of *Lactobacillus* spp Isolated from breast milk

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Breast milk is the combination of bioactive compounds and microflora that promote newborn's proper growth, gut flora, and immunity. Thus, it is always considered the perfect food for newborns.

However, it had been presumed to be germ-free, human milk now believed to contain many bacteria including. One of the most important genera of probiotic bacteria found in milk is *Lactobacillus*. The aim of the present study was to further analysis of *Lactobacilli* present in human milk.

### MATERIALS AND METHODS

47 milk samples were collected and cultured on MRS agar. The identity of isolates was further inspected using PCR reaction. The potential probiotic capacity was analyzed by the ability of the strains to withstand bile salt and acidic condition. Moreover, pathogen antagonistic effect of the isolates was assessed using agar spot test on ten prominent bacterial pathogens. To determine the nature of the pathogen killing mechanism of the isolated Cell free suspension was subjected to proteolytic enzymes and catalase enzyme.

### RESULTS AND DISCUSSION

754 bacterial colonies isolated from milk samples. 50 Out of 178 randomly-picked colonies were immune to acidic condition and Bile salt. 34 of these isolates were proved to *Lactobacillus* genus. Most of the isolated *Lactobacilli* were able to prevent the growth of pathogens. A major percentage of these isolated prevented the growth of the pathogen by producing organic acid.

### CONCLUSION

Consequently, these study suggests that human milk contains potential probiotic which may play a major role in infant's health Thus, the human-originated LAB strains are being furthermore characterized to exploit their probiotic and therapeutic potential towards specific human welfare in the form of customized functional foods and infant formulas.

**Keywords:** Human milk, Probiotic, *Lactobacillus*, Bacteriocin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation, Identification, and Optimization of L-asparaginase Production from *Enterobacter hormaechei* through Submerged Fermentation Using Response Surface Methodology

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

L-asparaginase, an enzyme responsible for converting L-asparagine into L-aspartic acid and ammonia has been employed as a chemotherapeutic agent for treating acute lymphoblastic leukemia. This enzyme's clinical effectiveness stems from its ability to reduce L-asparagine levels. Due to its therapeutic benefits, the discovery of microbial sources producing asparaginase has seen a significant increase in recent years. The objective of this study was to isolate and identify bacteria capable of producing the L-asparaginase from different samples and optimization of the influential parameters affecting its production.

### MATERIALS AND METHODS

The isolation of bacteria producing asparaginase from diverse samples such as soil, water, and dairy products was conducted using a specialized M9 medium enriched with phenol red and asparagine. Additionally, to assess the glutaminase activity in bacteria that exhibited a positive asparaginase test, a medium containing phenol red and glutamine was employed. The identification of bacterial isolates with positive asparaginase and negative glutaminase activity was achieved through a combination of morphological, biochemical, and 16S rRNA methods. Enzyme activity was measured using a colorimetric method. Then the factors affecting enzyme production such as carbon and nitrogen sources, temperature, and pH were optimized by Response Surface Method.

### RESULTS AND DISCUSSION

Out of the 19 enzyme-producing isolates screened on M9 agar, four were identified as excellent producers. Among these, only one isolate exhibited a lack of glutaminase activity. Analysis of the 16S rRNA sequences of these isolates revealed a remarkable 99.6% similarity with *Enterobacter hormaechei*. Finest enzyme activity was 6.78 IU/ml. Further, the L-asparaginase production was optimized using Response Surface Methodology (RSM). This approach led to the highest observed asparaginase activity, reaching 17.51 IU/ml. This optimal activity was achieved by utilizing glucose as the carbon source, ammonium chloride as the nitrogen source, temperature of 37°C, and the pH at 7.

### CONCLUSION

The study explored the *Enterobacter hormaechei* as a potent and potential bacterial source for high yield of anti-leukemic drug.

**Keywords:** L-asparaginase, *Enterobacter hormaechei*, Anti-leukemic drug, Optimization

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The role of the microbiome and its metabolites in cancer progression, regression and treatment

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

In spite of decades of clinical research, cancer remains a major public health burden and the leading cause of human mortality globally. In recent years, significant progress has been made in dealing with cancer, including early detection, diagnosis and treatment of cancer, and part of this progress is due to the attention paid to the gut microbiota. Until recently, the human microbiota was considered pathogenic, but today it has emerged as a vital player in maintaining human health. The microbiome is also known as the 'second brain' because of its significant pathophysiological role in human health and disease. Human gut microbes may increase, decrease, or have no direct effect on carcinogenesis. The use of the microbiota to optimise cancer treatments has become an alternative to personalised medicine.

### MATERIALS AND METHODS

A search was conducted on PubMed, Google Scholar, Magiran, and Web of Science databases from 2016 to 2023 using the terms "cancer therapy", "gut microbiota", "gut microbiota-derived metabolites", "immune system", and "Microbiota"

### RESULTS AND DISCUSSION

Gut microbes are undeniably potential candidates for predictive biomarkers and therapeutic targets. Some microbial metabolites have a direct carcinogenic effect. For example, colibactin from pks+ *E.coli* acts as a DNA alkylating agent, causing double-strand breaks and interstrand cross-links that destabilize the genome of human intestinal epithelial cells and ultimately lead to colon cancer. Besides carcinogenic effects, certain metabolites derived from microbiota possess anticancer functions. Butyrate produced by intestinal microorganisms increases the mRNA expression of claudins, thus effectively protecting intestinal epithelial cells from damage and preventing the development of colon cancer. Cancer development can be indirectly facilitated by microbes that stimulate inflammation or weaken immune surveillance. These microbial immunomodulatory activities are referred to as the "microbiome-immune-oncology axis".

### CONCLUSION

Considering the extensive role of microbiome and microbial metabolites in cancer, targeting them as a starting point for discovering new cancer treatment approaches will inevitably bring more benefits to cancer patients. However, more relevant experimental data are needed. Some microbial metabolites have cancer-causing and cancer-fighting effects. There are still several aspects of microbial metabolites that require further investigation.

**Keywords:** Gut microbiota, Gut microbiota-derived metabolites, Immune system, Microbiota

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Optimization of suitable carbon source for polysaccharide production by *Lactobacillus* sp. UTMC 3983

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Currently, exopolysaccharide is one of the most important used biopolymers. So, newly isolated strains with the ability to produce more exopolysaccharide such as *Limosilactobacillus* have become attractive fields of industrial microbiology research. The aim of this study was to propose different sources of carbon and examining different concentrations of these sources, to find the optimal carbon source.

### MATERIALS AND METHODS

In this study, the lactic acid strain of *Lactobacillus* sp. UTMC 3983 was used. Bacteria were inoculated on the Erlenmeyer flask containing the MRS broth culture (37°C, 100 rpm for 24hours). Then, 10% of the volume of seeding flasks was replaced with other carbon sources (including lactose, whey, molasses, glucose syrup, and sucrose) in Erlenmeyer flasks containing production based on MRS broth and polysaccharide production, residual substrate and bacterial biomass are measured.

### RESULT AND DISCUSSION

By examining the addition of 2.5% to 60% concentrations of sucrose to the culture medium and examining the amount of changes in exopolysaccharide production, the highest production rate was 67 g/liter at a concentration of 30%. By replacing the concentrations of (20-25-30-35-40)% whey as a carbon source, the amount of exopolysaccharide production in the highest concentration of whey (40%) was 12 g/liter. Also, by replacing the concentrations of (30-40-50-60)% lactose and molasses with concentrations of (20-30-40)% the amount of exopolysaccharide production was reported zero. By replacing the concentrations of (10-20-30-40) % of the glucose syrup, the average amount of exopolysaccharide production with the highest concentration (40%) was detected as 24.4 g/liter.

### CONCLUSION

The results of this study showed that the bacteria were unable to use lactose sugar, whey and molasses. By examining glucose syrup as a carbon source, it was also determined that this carbon source is only suitable for the bacteria revival, although exopolysaccharide production in this medium was weak. Finally, the medium with 30% sucrose (approximately 67 g/L) was found to be the most suitable carbon source for the growth and production of exopolysaccharide.

**Keywords:** Optimization, Exopolysaccharide, Lactic acid bacteria, *Limosilactobacillus*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Molecular typing of clinical isolates of *Mycobacterium tuberculosis* referred to the west tuberculosis center by PFGE method

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Tuberculosis (TB) is a highly contagious and chronic infectious disease. Nowadays, TB is causative a public health issue in the global. Molecular typing information is important role for ascertain the genotypic diversity of *M. tuberculosis* strains and to control infectious diseases transmission and in the prevention of the tuberculosis spread differentiation between new infections, and relapses that can effectively control the infections. The study aimed to compare the ability of restriction enzymes to detection the genetic relevance between strains and antimicrobial resistance profiles among *Mycobacterium tuberculosis* isolated from patients.

#### MATERIALS AND METHODS

Totally, 74 *M. tuberculosis* isolates were collected from patients referred to the tuberculosis center. Identification of all isolates was accomplished by standard biochemical methods. drug susceptibility test was performed using the commensurate method then the bacteria cells be embedded in a plug of agarose. PFGE plaques were digested by, *Xba*I, *Dra*I restriction enzyme. Finally, the digested DNA fragments were separated on 1% agarose gel and analyzed by Bio Numerics software.

#### RESULTS AND DISCUSSION

Thirteen (17.6%) out of 74 isolates were MDR, mostly related to Kermanshah patients; 59 isolates were digested with *Dra*I enzyme and 53 isolates with *Xba*I enzyme. The discriminatory power of *Dra*I (8 pulsotypes) is slightly higher than *Xba*I (4 pulsotypes).

#### CONCLUSION

Genotyping methods to demonstrate the spread of resistant *M. tuberculosis* isolates is important. The PFGE is considered as a powerful and useful tool to study the genetic diversity of many microscopic species. Therefore, this method is suggested as complementary techniques for molecular epidemiological studies.

**Keywords:** Molecular typing, *Mycobacterium tuberculosis*, Polymerase chain reaction, Pulsed-field gel electrophoresis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and characterization of probiotic bacteria isolated from cow milk

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Probiotics are live beneficial bacteria with positive effects on the host's health. These bacteria can eliminate harmful pathogens in the intestine and maintaining the balance of intestinal microbial flora. For example, *Lactobacilli acidophilus* are "friendly" bacteria that normally live in our digestive, urinary and genital systems without causing disease. Some of the functions of these bacteria include antimicrobial activity, improving body metabolism, anti-diarrheal properties, and improving inflammatory bowel diseases. Accordingly, the aim of this research was to isolate and identify of probiotic bacteria from milk samples.

#### MATERIALS AND METHODS

Samples of cow milk were collected from Kerman city (south east of Iran) during spring 2023. Samples in Falcon tubes in ice-box were delivered to the research lab. Milk samples serially diluted from  $10^{-1}$  to  $10^{-6}$ . 100  $\mu$ L of different were inoculated on MRS agar medium. Pured probiotic bacteria were characterized by different standard tests such as Gram staining, catalase, indole production, motility, and determination of fermentation patterns of sorbitol, arabinose, glucose, lactose, galactose, sucrose, and mannitol.

#### RESULTS AND DISCUSSION

MRS Agar was a medium for the cultivation and enumeration of *Lactobacillus* spp. and most lactic acid bacteria. Accordingly, out of 30 milk samples, 5 probiotic bacteria were isolated from *Lactobacilli* MRS Agar. Based on biochemical and diagnostic tests, all isolated strains were Gram-positive and catalase-negative. Four isolates were bacilli and the remaining one was coccus. Examination of indole production were negative, 4 isolates were non-motile. Three strains were able to ferment arabinose, and 2 ones were able to ferment sorbitol. None of them could ferment sucrose, galactose, or lactose. Additionally, all 5 bacteria were able to ferment glucose.

#### CONCLUSION

As consumers require foods not only providing energy but also with the ability to improve and enhance their health, probiotic dairy products are in a special importance. Above mentioned isolates may be behave as good candidate probiotics, but further *in vitro* and *in vivo* studies on these strains are still required and ongoing .

**Keywords: Probiotics, milk, isolation and chracterization**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation, Identification and Evaluation of activity and stability of Laccase Production by Halotolerant *Enterobacter* sp. GR18 Isolated from Grawan Mineral Spring

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

In recent years, enzymes have gained increased importance due to their applications in a variety of industries. Laccases are one of the most widely studied oxidoreductase enzymatic systems which are amply present in nature. They are widely explored as they can catalyze oxidization of a large range of phenolic and aromatic compounds. The objective of this study was to isolate, identify and investigate the activity and stability of laccase produced by a halotolerant bacteria isolated from Grawan mineral spring.

### MATERIALS AND METHODS

The isolation of bacteria producing Laccase from diverse samples such as water, sludge, and soil was conducted using MHM medium. The identification of bacterial isolates with positive Laccase activity was achieved through a combination of morphological, biochemical, and 16S rRNA methods. Enzyme activity was measured using a colorimetric method. Finally, the effect of various physic-chemical factors such as carbon and nitrogen sources, metal ions, inducers, incubation time, temperature and pH on the enzyme stability and activity was evaluated.

### RESULTS AND DISCUSSION

Out of the 5 enzyme-producing isolates screened on MHM medium, one was identified as excellent producer. Analysis of the 16S rRNA sequences of these isolates revealed a remarkable 99.6% similarity with *Enterobacter hormaechei*. Results showed that glucose and yeast extract are the optimal sources of carbon and nitrogen, respectively. Cu<sup>2+</sup> and bis-phenol emerged as the best cation and inducer. Optimal laccase activity was achieved at a pH of 6 and a temperature of 30°C, following a 96-hour incubation time.

### CONCLUSION

Findings of this study suggest that the Grawan mineral spring serves as a valuable resource for isolating robust microorganism capable of producing economically significant enzymes, thereby facilitating cost-effective industrial applications.

**Keywords:** Laccase, *Enterobacter hormaechei*, Stability, Grawan mineral spring

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Cloning and expression of aldehyde reductase in E.coli

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Aldehyde reductase is an enzyme that catalyze the NADPH-dependent reduction of a variety of carbonyl compounds and are widely distributed in mammalian and also in prokaryotes like E.coli. they are attractive biocatalysts for industrial applications like in food, pharmaceutical industry and fine chemical industry. Objective of this project is to clone and express the gene that encodes the aldehyde reductase enzyme in E.coli.

#### MATERIALS AND METHODS

In this project, the gene coding for the aldehyde reductase enzyme of E.coli has been amplified with specific primers(aldehyde reductase-SalI-R,aldehyde reductase-NcoI-F). The sequence digested using restriction enzymes (SalI,NcoI) that was already designed in the primers .Then the PCR product cloned into the pET26b-Yiat vector. The cloning of this fragment in the vector was confirmed by direct colony PCR And digestion with restriction enzymes SalI and NcoI. The recombinant vector was transformed into a suitable expression host (E. coli Rosetta (DE3)) and the resulting colonies were analyzed for expression and activity.

#### RESULTS AND DISCUSSION

The cloning of the gene Encoding the aldehyde reductase enzyme fragment was confirmed by PCR and agarose gel electrophoresis. Restriction digestion pattern of recombined construct confirmed also. Colonies that were induced by 1mM IPTG showed biological activity compared to the control sample. Cloning the aldehyde reductase gene under the T7 promoter and in the correct expression frame resulted in its expression in the host bacteria. This expression confers the reducing activity to the host, which can reduce NAD to NADH in the recombinant host cell. Optimizing the expression of aldehyde reductase enzyme in this host using different culture conditions is in progress.

#### CONCLUSION

According to the previous explanations about the role of aldehyde reductase in various industries, biologically production of this enzyme is of great importance considering its advantages, and more efforts and research should be done in the field of optimizing its performance.

**Keywords:** aldehyde reductase, biocatalysis, cloning, Escherichia coli, gene expression

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# First assessment of SEN virus in hemophilia patients: A high prevalence in comparison to healthy population

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Hemophilia and transfusion dependent patients are at high risk of blood born viruses including viral hepatitis and non-A non-B agents. SEN virus (SEN-V) is a newly identified blood born virus that is associated with transfusion-induced non-A to E hepatitis. Therefore, this study aimed to investigate the prevalence of SEN-V in hemophilia patients in association to complications and risk factors

### MATERIALS AND METHODS

This was a cross sectional case-control study conducted in Hemophilia Center in east of Iran, South Khorasan Province. Blood samples were taken from patients and healthy control population; next demographic and clinical information were taken. The sera samples were subjected for DNA extraction, then SEN-V and its genotype were detected by PCR-based methods and phylogenetic analysis was performed. The collected data were analyzed and interpreted by SPSS22 software.

### RESULTS AND DISCUSSION

The mean age of patients was  $26.18 \pm 14.97$  and the healthy age was  $41.69 \pm 14.05$ . Among the patients and healthy group, 94.5% and 36.4% were male, respectively; the rest were female. Most cases in the patient group had hemophilia type A (85.5%), then type B (7.3%) and VWD type (3.6%) and F and plt type (1.8%) were in the next categories. SEN-DNA was detected in 58.2% of patients and 20% of healthy groups (P-value: 0.00). Among these, H and D genotypes were found in 59.4% and 40.6% of patients and 63.6% and 36.4% of healthy groups, respectively. The prevalence of the virus was higher in hemophilia A (63.8%) and its severe type was much higher (63.2%).

### CONCLUSION

The results of this study showed a very high prevalence of SEN virus in hemophilia patients compared to healthy individuals. These results indicate the need for monitoring and follow-up of this high-risk group in terms of blood-borne pathogens.

**Keywords:** Hemophilia, SEN Virus, Prevalence, Blood Born Pathogens, Hepatitis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of urinary tract infection and risk factors related to it and evaluation of antibiotic resistance in pregnant women of Zahedan city in 1401

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Urinary tract infection is the most common bacterial infection during pregnancy and an important risk factor for severe maternal and perinatal outcomes. This study was conducted with the aim of determining the prevalence of urinary tract infection and its related risk factors.

### MATERIALS AND METHODS

The present study was conducted on the mid-morning urine of 200 pregnant women suspected of having a urinary tract infection who had referred to the treatment center of Zahedan hospitals. For each sample, complete urine and culture tests were performed. Then, the isolated bacteria were colonized with more than 100,000 bacteria per milliliter of anti-biogram.

### RESULTS AND DISCUSSION

In this way, the obtained results showed that 29 samples (14.5%) were positive out of 200 urine samples tested. The most common clinical findings were fever (72.42%), heartburn (41.30%), flank pain (6.9%) and blood in urine (6.9%). The most common microbes in positive urine cultures are: 16 cases of *Escherichia coli* (57.14%), 3 cases of *Klebsiella* (10.41%), 2 cases of *Pseudomonas* (4.16%), one case of *Proteus vulgaris* and one case of coagulase-negative *Staphylococcus* each (3.57%) and 4 cases were caused by mixed bacteria (14.29%). The results of the anti-biogram test showed in terms of bacteria. The degree of sensitivity of *E.Coli* It was resistant to gentamicin (26/79) and to gentamicin, tobramycin and ciprofloxacin (33/8) and to cotrimoxazole (65/72). *Klebsiella* was sensitive to gentamicin (82/75) and ciprofloxacin (72/41) and resistant to cotrimoxazole (59/26).

### CONCLUSION

Proper spacing between pregnancies, special care of pregnant women of middle to low social class and women suffering from severe vomiting of pregnancy can play a significant role in preventing urinary tract infections and related complications. The results of this study indicate a significant increase in antibiotic resistance among urinary tract infection agents. Based on the findings of the research, it is recommended to be more careful in choosing and prescribing antibiotics for experimental treatment, and the antibiotic sensitivity pattern of the disease-causing bacteria must be investigated before prescribing the drug.

**Keywords:** Urinary Infection, Antibiotic Resistance, Pregnant Women.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### ***In-vitro* Effect of Meropenem, Colistin, and Amikacin Combination against Carbapenem-resistant and Biofilm-forming *Klebsiella pneumoniae***

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#### **ABSTRACT**

#### **BACKGROUND AND OBJECTIVES**

The present study was aimed to investigate *in-vitro* inhibitory effects of meropenem, colistin, and amikacin alone and the various combinations against carbapenem-resistant and biofilm-forming *Klebsiella pneumoniae*.

#### **MATERIALS AND METHODS**

Ten carbapenem-resistant and biofilm-forming *K. pneumoniae* isolates were isolated from clinical specimens. The resistance to carbapenem were determined by determination of meropenem minimum inhibitory concentration (MIC) by the agar dilution methods according to the Clinical & Laboratory Standards Institute (CLSI) guideline. To study any inhibitory effect of antimicrobial agents on biofilm, the minimum biofilm inhibitory concentration (MBIC) was determined. The synergetic effect of the antibiotics combinations was studied using the checkerboard assay and the fractional inhibitory concentration (FIC).

#### **RESULTS AND DISCUSSION**

The highest synergic effect against planktonic form was observed in meropenem/amikacin (8 of 10 isolates), and the lowest synergic effect was found in amikacin/colistin (2 of 10 isolates). Colistin/meropenem were shown synergic effect for 5 isolates. The highest synergic effect against biofilm was observed in colistin/meropenem (7 of 10 isolates) followed by meropenem/amikacin (3 of 10 isolates) and colistin/amikacin (2 of 10 isolates).

#### **CONCLUSION**

The combination of antimicrobial agents had shown the different effects on biofilm and planktonic forms of *K. pneumoniae*. Therefore, a separate determination of inhibitory effects of the antibiotic in the combination is necessary for biofilm forms. amikacin/meropenem was more effective against planktonic and colistin/meropenem against biofilm forms of *K. pneumoniae*.

**Keywords:** Carbapenem, Biofilm, *K. pneumoniae*, Synergic effect

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Carbapenems resistant *Enterobacteriaceae* isolated from wounds infections from Tabriz, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

This study was aimed to investigate antibiotic susceptibility patterns and carbapenem resistance mechanisms of *Enterobacteriaceae* isolated from wound infections of Tabriz during 2021-2023.

### MATERIALS AND METHODS

Two hundred and five *Enterobacteriaceae* isolated was isolated from wound infections. The disk diffusion and microbroth dilution methods were applied for determine the antibiotics susceptibility patterns. Carbapenemase mediated resistance mechanisms were detected by modified carbapenem inactivation method (mCIM). The carbapenemase genes were detected by the PCR method.

### RESULTS AND DISCUSSION

According to the antimicrobial susceptibility testing, a high level of resistance was observed to different class of antibiotics except colistin and amikacin. Among carbapenem resistant isolates, *Klebsiella pneumoniae* was most common (39/61; 63.93%) followed by *Escherichia coli* (18/61; 29.50%), and *Enterobacter spp* (4/61; 6.55%). AmpC overexpression was detected in all *Enterobacter spp* isolates. According to the mCIM and PCR results, *E. coli* and *K. pneumoniae* were carbapenem resistance due to carbapenemase production. The most common carbapenemase gene was bla<sub>OXA-48</sub>-like (49.12%) followed by bla<sub>KPC</sub> (26.31 %) bla<sub>NDM</sub> (21.05 %), and bla<sub>VIM</sub> (3.5%).

### CONCLUSION

The frequency of carbapenems resistant *Enterobacteriaceae* in our setting is at a worrying level. The most common mechanism of resistance to carbapenems was carbapenemase. We suggest revision in the controlling program of wound infections caused *Enterobacteriaceae* in our hospitals.

**Keywords:** Wound infections, Carbapenem, *Enterobacteriaceae*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Colistin susceptibility in Carbapenem resistant *Enterobacteriaceae* isolates from wound infections, Tabriz, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The present study was aimed to study colistin susceptibility in Carbapenem resistant *Enterobacteriaceae* (CRE) isolated from wound infections.

### MATERIALS AND METHODS

Two hundred and five *Enterobacteriaceae* were isolated from wound infection during 2021-2023. The disk diffusion assay was performed according to the Clinical and Laboratory Standards Institute (CLSI)- established breakpoints. The colistin and meropenem MIC (Minimum Inhibitory Concentration) was determined by the micro-broth dilution according to CLSI guidelines.

### RESULTS AND DISCUSSION

Among the 205 *Enterobacteriaceae*, 61 isolates (29.75%) were CRE included *Klebsiella pneumoniae* (39/61; 63.93%) followed by *Escherichia coli* (18/61: 29.50%), and *Enterobacter spp* (4/61; 6.55%). All carbapenem resistant isolates were MDR. The colistin MIC range was 0.12 to 16 µg/mL. Two *K. pneumoniae* isolates (5.1%) were colistin resistant. The colistin MIC<sub>50</sub> and MIC<sub>90</sub> were 0.25 and 1 µg/mL, respectively.

### CONCLUSION

Colistin may be an alternative antimicrobial agent for infections due CRE. However, Colistin susceptibility should be studied before its usages in antimicrobial therapy.

**Keywords:** Carbapenem, Colistin, *Enterobacteriaceae*, Multi- Drug Resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### BCG vaccine in bladder cancer treatment

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Bladder cancer is the most common tumor in the urinary system, and muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC) are two main forms of it. In addition to the primary application of the BCG vaccine against tuberculosis in children, it is a crucial part of the treatment for NMIBC. In terms of microbial cancer treatments, this vaccine is undoubtedly the most successful case to date when it comes to treating bladder cancer.

#### MATERIALS AND METHODS

For this review, keywords such as bladder cancer, BCG vaccine, and treatment were searched in the titles of articles to find the best and newest ones. Out of 992 results based on reliability, 15 were selected.

#### RESULTS AND DISCUSSION

In patients with high-risk NMIBC, BCG maintenance treatments have been shown to be clinically beneficial in several trials. These therapies are still seen as having positive therapeutic effects, particularly in terms of preventing the recurrence of NMIBC. The most common side effects include dysuria, and urinary frequency.

#### CONCLUSION

The most commonly used method of treatment for patients with intermediate- and high-risk NMIBC is BCG, which decreases the likelihood of cancer progression and recurrence and probably increases overall survival. Current data suggests a complicated interaction between innate immune cells, T cells, and cancer cells. Our comprehension of the variables affecting clinical response keeps getting better as we clarify the mechanism of BCG. By designing more potent BCG strains or by combining BCG with other treatments in a synergistic manner, these insights will enable us to better adapt the initial NMIBC treatment decision to the patient and enhance the BCG treatment of bladder cancer.

**Keywords:** BCG vaccine, treatment, cancer, bladder cancer.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Designing and characterization of a nanoprobe for the specific detection of *Listeria monocytogenes*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Listeria monocytogenes* is a major foodborne pathogen causing listeriosis. Therefore, rapid detection of the bacteria with high accuracy is in demand and now possible with the development of genosensors. For this purpose, a biosensor based on a nanoprobe attached to a gold nanoparticle was designed for colorimetric-based detection.

### MATERIALS AND METHODS

*L. monocytogenes* was purchased from Persian Type Culture Collection (PTCC:1783). The Htr gene essential for tolerance of osmotic stress and facilitates growth of *L. monocytogenes* was selected as a specific gene by the whole genome analysis in NCBI nucleotide. Then, BLASTn was performed to determine its specificity. In order to design the probe, the value of GC ratio, T<sub>m</sub>, ΔG of the sequence and the possible secondary structures of the selected oligonucleotide were considered. Au nanoparticles were synthesized using citrate reduction method with the mean size of 20-30 nm. The nanoprobe was functionalized using a reducing agent and gradual increase in ionic strength using PBS (pH, 7). The Au nanoparticles and Au nanoprobe were subjected to optical spectrometry and DLS tests to verify the required properties. The stability of the Au nanoprobe was measured against Au nanoparticles by an increase in salt concentrations.

### RESULTS AND DISCUSSION

The BLAST results of the probe revealed 100% specificity of the designed oligonucleotide for *L. monocytogenes* detection. A probe with a length of 26 bp was designed. The GC ratio of the probe was 42.3% and the T<sub>m</sub> and ΔG were 65°C and -36.6 kcal/mol, respectively. DLS analysis of the gold nanoparticles showed a mean diameter of around 39 nm while the mean size of Au nanoprobe was 60 nm. The nanoprobe color remained stable after magnesium chloride salt induction, the nanoparticles were totally precipitated and transformed into a blue color. This represents the accurate functionalization process and high stability of Au nanoprobe in comparison with Au nanoparticles. The absorption peak of the nanoprobe was 0.094 at 520 nm, however, Au nanoparticles had an absorption of 0.071.

### CONCLUSION

We have developed a nanoprobe specific for the detection of *L. monocytogenes* which could be used for biosensor or detection kit establishment. In terms of economic efficiency and time management, it is crucial to apply the nanoprobe to a detection method with high sensitivity and speed.

**Keywords:** *Listeria monocytogenes*, biosensor, foodborne pathogens, gold nanoparticles, nucleobiomarkers.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation of the antibacterial properties of Malva plant against standard and resistant hospital isolates of *Staphylococcus aureus* and *Klebsiella pneumoniae*

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

The emergence of microbial resistance seems inevitable with almost any new drug. Although antimicrobial resistance is a natural process, it is often the result of inappropriate use of drugs and treatment of infections. Natural extracts, such as plant extracts, have shown significant progress in the discovery of new antimicrobial compounds. The aim of this study is to investigate the antimicrobial effects of Malva extract against MDR and standard *Staphylococcus aureus* and *Klebsiella pneumoniae* bacteria.

### MATERIALS AND METHODS

The alcoholic extract of the Malva plant was followed by MIC and MBC tests against two MDR and standard isolates of *Klebsiella pneumoniae* and *Staphylococcus aureus*.

### RESULTS AND DISCUSSION

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for standard *Staphylococcus aureus* were 3 mg/ml and 1 mg/ml for *Klebsiella pneumoniae*, respectively. The MIC and MBC were 2 mg/ml and 1 mg/ml for MDR *Staphylococcus aureus* and 1 mg/ml for MDR *Klebsiella pneumoniae*, respectively.

### CONCLUSION

Plant extracts with antimicrobial compounds can be used to destroy bacteria that are resistant to all types of antibiotics, significantly reducing the prevalence of antibiotic resistance.

**Keywords:** Antibacterial, Malava, *Staphylococcus aureus*, *Klebsiella pneumoniae*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The potential of CRISPR/Cas9 technique in bacterial epigenome engineering

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The CRISPR/Cas9 system has emerged as a revolutionary genome editing tool, primarily known for its ability to precisely modify DNA sequences. However, its potential extends beyond genome editing to the realm of epigenetics. Bacterial epigenome engineering, encompassing modifications of DNA methylation patterns and the manipulation of non-coding RNA molecules, holds promise for advancing our understanding of gene regulation and cellular processes. This paper aims to explore the untapped potential of CRISPR/Cas9 in bacterial epigenome engineering.

Epigenome engineering in bacteria and the role of CRISPR/Cas9:

The bacterial epigenome plays a crucial role in the regulation of gene expression, cellular differentiation, and stress responses. Traditional techniques for studying bacterial epigenetics often lack specificity and precision. However, the unprecedented programmability of the CRISPR/Cas9 system offers a revolutionary approach to selectively target and modify the bacterial epigenome. This system allows us to precisely control the gene expression and elucidate the impact of specific methylation patterns on bacterial phenotypes. Furthermore, CRISPR/Cas9 can be exploited in the emerging field of RNA-based epigenome engineering, where this system can target non-coding RNA molecules and investigate their regulatory roles in the bacterial cells. By harnessing the versatility of CRISPR/Cas9 technology, researchers can probe the functions of non-coding RNAs, uncover new regulatory mechanisms, and potentially develop RNA-based therapeutic interventions.

Challenges:

Despite the tremendous potential of CRISPR/Cas9 in bacterial epigenome engineering, several challenges remain. These include off-target effects, the need for efficient delivery methods, and ethical considerations. In eukaryotes, epigenetic modification of the genome involves DNA methylation and histone modification. Bacteria lack histones, and epigenetic control relies on DNA methylation only. In both bacteria and eukaryotes, transcriptional repression by DNA methylation is common. Transcriptional activation of bacterial genes under DNA methylation control often involves demethylation (partial or complete, single- or double-stranded) of promoters or regulatory regions.

#### CONCLUSION

In conclusion, the CRISPR/Cas9 system offers an exciting avenue for exploring and engineering the bacterial epigenome. By expanding our understanding of bacterial epigenetics, this technology opens new possibilities for therapeutics, bioengineering, and the advancement of fundamental biological knowledge. Continued research in this field will undoubtedly uncover fascinating insights into the complex interplay between the epigenome and cellular processes in bacteria.

**Keywords:** Epigenome modifications; CRISPR-Cas system; Epigenome engineering; Bacterial epigenetics

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The Antibacterial Effect of Aqueous extract from Gonads of Sea Urchin *Echinometra mathaei* on *Helicobacter pylori*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Oceans cover 70% of the Earth's surface. In recent decades, research on chemicals derived from marine organisms with high bioactivity has made marine life a bountiful source of metabolites that are beneficial to human health and quality of life. The primary objective of this study was to assess the antimicrobial efficacy of aqueous extracts obtained from the sea urchin *Echinometra mathaei*, gonads. The target microorganisms for evaluation were *Helicobacter pylori*, which has faced difficulties in its treatment in recent years due to the increase in antibiotic resistance.

#### MATERIALS AND METHODS

The sea urchins were taken from the Boushehr coastal region, during the year 2021. The gonads of sea urchins rinsed with tap water, and then separated for the purpose of extraction. The tissue was homogenized for 5 min. Samples were shaken for 24 h (90 rpm) and centrifuged at 12000 rpm for 12 min at 4 °C. The supernatant was kept at 4 °C, and the residue was centrifuged again. After centrifugation and freeze-drying, the supernatants were stored at -70 °C until antibacterial testing. This study investigated the inhibition zone and minimum inhibitory concentration (MIC) values of sea urchins extract against *H. pylori* utilizing seven clinical isolates using an in vitro disc diffusion and agar dilution assay according to the CLSI standard.

#### RESULTS AND DISCUSSION

Following a 72-hour incubation period at a temperature of 37°C and microaerophile atmosphere, The aqueous extracts (8 mg/ml) of sea urchins do not inhibit the growth of *H. pylori* in vitro, no inhibition was seen. *H. pylori* was inhibited in an agar dilution assay with minimal inhibition concentration (MIC) 1000 µg/mL. This finding suggests that the compounds in of sea urchin gonads are effective in vitro against *H. pylori*.

#### CONCLUSION

This research demonstrated that *E. mathaei*, often known as the sea urchin, has the potential to provide new classes of antibiotics. The extract from sea urchins, which have been shown to suppress *H. pylori* growth, should be further studied to isolate, purify, and identify the chemical structure of the antibacterial chemicals for medical application.

**Keywords:** Persian Gulf Sea Urchin, *Echinometra mathaei*, Antibacterial effects, Gonad, *Helicobacter pylori*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Biom mineralization Function in Conservation of Calcareous Stone of Built Heritage: A perspective to the further investigations

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### ABSTRACT

Calcareous stones are one of the most widespread material in build heritage artifacts all around the world, and in particular, in Iran. Biom mineralization is a common phenomenon in the nature which divided into authigenic and artificially induced mineralization. In two recent decades, bacteria-induced mineral precipitation based on the natural phenomenon, has been gradually extended to an innovative method to consolidate the stone of cultural heritage. Carbonatogenesis (biocalcification) is a term of bacterial mineralization of calcium carbonate that the carbonate productivity is strongly dependent on the mineralogy of the substrate. The aim of this study is introducing this new method and its advantage and limitation to the researchers in the field of microbiology who are interested in the cultural heritage materials. The Recent studies on biom mineralization proved this method has too many advantages that the most important one is compatibility, and then, it has more advantages such as sealing the microcracks through biological carbonate, decreasing the porosity that results reduction of water absorption as well as providing an appropriate eco-friendly method. But, the depth of penetration of the cementing carbonate is often limited to less than a millimeter, also, some treatments create a sacrificial layer rather than substantial penetration. It is known, that the microbes can strongly contribute to stone deterioration, therefore, changes in the stone microbial community structure and growth of unwanted microorganisms could be a risk factor. In general, there are some factors that can influence on the results including particle size, solution ion content, grouting method, substrate concentration, temperature, pH, etc. In addition, there is a relationship between the quality of the calcium carbonate produced and the oxygen content in the environment, thus, microbial induced calcium carbonate precipitation from laboratory to field application is very different and needs more in situ investigations. In addition, the possibility of undesirable side-effects and risks to the stone that needs to be carefully evaluated.

**Keywords:** Biom mineralization, Calcareous Stone, Built Heritage, Carbonatogenesis,

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation and characterization of pectinase-producing bacteria from fruit and vegetable soil

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Pectinolytic bacteria are a type of bacteria that can break down pectin, a complex polysaccharide that forms a vital component of plant cell walls. These bacteria secrete enzymes called pectinases that facilitate the efficient degradation of pectin into smaller molecules. Pectinase can be found in fungi and bacteria. Pectinases play a crucial role in fruit juice extraction and the modification of food product texture.

### MATERIALS AND METHODS

Soil samples were collected from decaying fruits, including apples, oranges, potatoes, and vegetables. The samples were suspended in nutrient broth medium and inoculated onto nutrient agar. The cultures were incubated overnight either at RT, 37°C and/or 4°C. After observing the morphology of colonies, they were sub-cultured onto other nutrient media to isolate individual colonies. Then, morphological and microscopic characteristics of isolates were analyzed. To test their pectinolytic activity, we transferred them to pectin agar media containing only pectin as a carbon source. After incubation, a diluted solution of iodine-potassium iodide was applied to determine the size of the clear zone that surrounded the colonies.

### RESULTS AND DISCUSSION

From soil samples, 20 pure isolates were obtained and characterized. It was revealed that out of the total isolates, twelve colonies were gram-positive while eight were gram-negative. Additionally, 12 isolates were found in round-shaped cocci and 8 isolates were in the form of rod-shaped bacilli. All 20 isolates were grown on a pectin agar medium. Upon adding diluted iodine-potassium iodide solution to the medium and observing the clear zone formed around the colonies, five isolates represented the highest pectinolytic activity and were chosen for further investigation.

### CONCLUSION

Identifying isolates with high pectinolytic activity is crucial for various industries that rely on pectinase enzyme production. Therefore, a thorough study is needed identify potential sources. We have isolated and characterized the pectinase-producing bacteria with higher pectinase activity and subjected to further molecular identification, characterization, and optimization.

**Keywords:** Industrial enzymes, Pectinolytic bacteria, Pectinase, Pectin, Iodine-potassium iodide

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The use of recombinant *Francisella tularensis* antigens for tularemia serodiagnosis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Tularemia is an important zoonosis caused by *Francisella tularensis*. The serological tests such as enzyme-linked immunosorbent assay (ELISA), are widely used for tularemia diagnosis. The classical ELISA tests rely on lipopolysaccharides (LPS) of the bacterium as antigens, which requires culturing live strains of *F. tularensis* in the laboratory and can lead to false-positive results due to cross-reactions with other gram-negative bacteria. To address these limitations, the researchers propose using recombinant proteins as antigens for tularemia serodiagnosis. This study aimed to identify immunodominant antigens of *F. tularensis* that posed strong immunological properties and diagnostic value based on a literature review and conducting a bioinformatics analysis on them.

### MATERIALS AND METHODS

We conducted a literature review among the articles in PubMed and Scholar. In this research, immunodominant antigens that posed strong immunological properties and diagnostic value were selected among the studies. Finally, six proteins including FTT0077 (2-oxoglutarate dehydrogenase component E2, succinyltransferase dihydrolipoamide), FTT0975 (conserved hypothetical protein), FTT0975 (conserved hypothetical protein), FTT1696 (chaperone 60 (GroEL; 57 kDa), FTT1269c (DnaK chaperonin), FTT0583 (FopA (outer membrane associated protein) and FTT0472 (Acetyl-CoA carboxylase, biotin carboxyl carrier protein subunit) that were more immunodominant selected for this purpose. Then, bioinformatics studies were conducted to assess their heterogeneity, indicating that these genes are conserved.

### RESULTS AND DISCUSSION

The present study identified six immunodominant *F. tularensis* antigens through proteomics, microarray, and immunoblotting techniques including FTT0077, FTT0975, FTT1696, FTT1269c, FTT0583, and FTT0472 based on literature review. The selected antigens, including FTT0077, FTT0975, FTT1696, FTT1269c, FTT0583, and FTT0472 showed a heterogeneity rate ranging from <0.63 to <0.31, indicating that they are conserved genes and have the potential as diagnostic targets. The use of these recombinant proteins in the diagnosis of tularemia is suggested as they are more immunodominant and elicit an immune response.

### CONCLUSION

This study suggests that using these six recombinant proteins in the diagnosis of tularemia can overcome the limitations of current methods and provide a new strategy for accurate and early detection of the disease. Further research and validation studies are needed to establish the effectiveness and reliability of these proteins in the diagnosis of tularemia.

**Keywords:** *Francisella tularensis*, Recombinant proteins, Serological diagnosis, Tularemia

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Pattern of macrolide resistance in *Clostridium perfringens* isolates from small ruminants in Kerman, Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Clostridium perfringens* (*C. perfringens*) is an anaerobic spore-forming Gram-positive bacteria, ranking amongst the most important pathogens living in the intestinal tracts of both humans and animals. Its pathogenicity is largely attributed to produce several potent toxin and several extracellular enzymes which can cause histotoxic infections, food poisoning and a spectrum of intestinal diseases like enteritis and enterotoxaemia. Enterotoxaemia is a pathological condition characterized by the absorption of toxins produced within the intestinal tract, which ultimately leads to deleterious effects on several internal organs, including the brain, kidneys, lungs, and heart. It is one of the most frequently reported diseases of sheep and goats all over the world. In different countries, the prevalence rates of the enterotoxaemia ranges from 24.13 to 100% in small ruminants. Antimicrobial agents are used for control and treatment of this disease and sometimes clinical outbreaks do not respond well to certain treatments. The objective of this study was to determine the resistance of *C. perfringens* isolates to macrolide antibiotic class.

#### MATERIALS AND METHODS

This study was performed on a total of 273 *C. perfringens* isolates, which were previously recovered from fecal samples of the diseased small ruminants in Kerman province, Iran. They were evaluated for the antimicrobial resistancy against tylosin and erythromycin (macrolide class) antibiotics by Kirby-Bauer disk diffusion method.

#### RESULTS AND DISCUSSION

The findings of our study demonstrated the high antibiotic resistant rate of *C. perfringens* isolates in small ruminants. The highest resistancy were observed to tylosin (53.11%), and erythromycin (23.44%) antibiotics, respectively.

#### CONCLUSION

To the best of our knowledge, this is the first study regarding the antimicrobial susceptibility of *C. perfringens* in small ruminants in Kerman province of Iran. This pattern of antibiotic resistance in *C. perfringens*, potentially reflecting the farm usage of these agents. Tight restriction of unnecessary antibiotic uses is necessary for some clostridial diseases.

**Keywords:** *Clostridium perfringens*; Macrolide; Tylosin; Erythromycin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation of BTEX-decomposing bacteria from Lordegan petrochemical effluent, Behregan oil-contaminated soil and Isfahan refinery oil sludge

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

BTEX is one of the most important environmental pollutants. In this study, we succeeded in isolating 2 bacteria, *Bacillus sp* and *Arthrobacter pascens*, which had high growth ability in the presence of 1% BTEX and can be used for the biodegradation of BTEX.

Today, the biodegradation method by bacteria is the most effective method to remove BTEX from contaminated sites. The aim of this study is to isolate bacteria with high growth ability in the presence of BTEX, which can be used for BTEX biodegradation.

### MATERIALS AND METHODS

In this research, solid and liquid petrochemical samples of Lordegan, oil contaminated soil samples and oil sludge samples were collected. Then, BTEX-decomposing bacteria were isolated and purified by chemotaxis method in MSM culture medium containing BTEX compounds. Next, bacteria were cultured in liquid MSM culture medium containing 1% BTEX as the only carbon source and their ability to grow in the presence of BTEX was checked through OD<sub>600</sub> reading. Finally, 2 selected strains were identified by 16S rRNA gene sequencing.

### RESULTS AND DISCUSSION

The isolated strains are capable of high growth in the presence of 1% BTEX. Based on molecular identification, these strains were 99% similar to *Bacillus sp* and the strain was 98.36% similar to *Arthrobacter pascens* bacteria.

### CONCLUSION

Many studies in the world confirm the positive effect of bacteria in the biodegradation of BTEX compounds. As a result, *Bacillus sp* and *Arthrobacter pascens* bacteria can be used to remove BTEX compounds from the environment.

**Keywords:** Benzene, Toluene, Ethylbenzene, Xylene, Biodegradation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Phenotypic isolation and screening dominant bacteria causing spoilage in dairy products

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Microbial contamination by food products could cause serious digestive and enteric diseases. Various methods exist for the identification and characterization of microorganisms. In this study we aimed to screen and isolate different types of bacteria that grow in dairy products for detecting the dominant spoiling agents.

### MATERIALS AND METHODS

The samples including milk and drinkable yogurt (Dough) were provided by one of the dairy companies. Enriched cultivation environment was applied using tryptic soy broth (TSB) to isolate all the bacteria in the products. After overnight incubation, we transferred the bacteria from broth culture to the tryptic soy agar (TSA) using pour plate method. Then streak plate method was used to obtain single colonies. The colonies were characterized individually using morphological analysis and phenotypic examination of the bacteria by Gram staining and microscopic investigation. Universal primers were designed and synthesized for 16S rDNA molecular PCR-based analysis.

### RESULTS AND DISCUSSION

Eight distinct dominant single colonies were obtained from milk and three from drinkable yogurt samples. The morphology of two colonies out of three in Dough sample were rod-shaped bacilli and one was round-shaped cocci. Six out of eight isolated colonies in milk were rod-shaped bacilli while two colonies were round-shaped cocci. All of the dominant colonies from Dough sample seem to be gram negative. Gram staining can show us that 75% of colonies in milk were gram negative and 25% of them were gram positive. The genomic DNA of the respective colonies were extracted and subjected to amplification. A distinct band of around 1550 bp was observed in all samples. Further molecular analysis to detect the genus and species of the bacteria is in progress.

### CONCLUSION

The main concept of this study was to identify the core bacteria causing the dairy to spoil before being used in the industry for making further products. By means of the information on dominant pathogenic bacteria, the company could prepare diagnostic kits and treatments to avoid early contamination and spoilage of products.

**Keywords:** Food microbiology; dairy products; molecular identification; spoiling agents; 16S rDNA

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### A new insight into the role of IL-17 in the severity of Covid-19

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

Interleukin 17 (IL -17) is a pro-inflammatory cytokine produced mainly by T helper type 17 (Th17). It plays an important role in generating a protective immune response against microbial infections by helping to deliver neutrophils to the site of infection. However, IL17 is considered a double-edged sword in some studies, suggesting that overexpression of IL -17 can cause overwhelming inflammation leading to acute tissue injury. The immune response is different in each individual. Single nucleotide polymorphisms (SNPs) in genes related to immune response cause genetic variations that affect susceptibility to various infections. Polymorphism can affect cytokine production and increase the severity of infection. Based on the aforementioned research studies, IL17 polymorphism may make a significant difference in the host immune response to Covid-19 infection.

##### MATERIALS AND METHODS

Publications accessible in international databases (Google Scholar and PubMed) were analyzed, searching for keywords such as "Covid-19", "SARS-CoV-2", "Interleukin-17" and "Polymorphism".

##### RESULTS AND DISCUSSION

Most studies have shown that the production of interleukin 17 is very important for the human immune response to infection. In a research study in 2021, an anti-IL-17 therapy was performed on 88 patients infected with Covid-19. After 3 days, patients' CRP and body temperature improved.

Another study investigated the IL -17 serum level in patients with severe acute respiratory syndrome and corona virus-2 (SARS-CoV-2). The results showed that overexpression of IL -17 can attract more inflammatory cells to the airways and cause tissue damage.

##### CONCLUSION

Most of the studies on IL -17 polymorphism have shown that IL -17 plays an important role in the severity of some infections and its overexpression may have deleterious effects leading to tissue damage in some diseases. Therefore, further studies on different IL -17 polymorphisms as a risk factor for Covid-19 infections are needed to find a solution to reduce the risks.

**Keywords:** Covid-19, IL-17, Th17, Cytokine, SNPs

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Designing HBHA-Omp25 recombinant protein with the aim of developing a vaccine against *Brucellamilitensis* bacteria

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Brucellosis is a common disease between humans and animals, which is endemic in most regions of developing countries and still causes economic losses and infects humans every year despite vaccination in cattle. Therefore, the purpose of this study is to design and investigate the recombinant structure of HBHA-Omp25 using bioinformatics method, so that a suitable alternative can be introduced for common vaccines that use live and attenuated bacteria.

#### MATERIALS AND METHODS

In this research, the design of the HBHA-Omp25 gene fragment and its specific primers was carried out using dedicated software and in-computer. In order to evaluate the immunogenicity score of the recombinant structure and its physicochemical properties, Vaxijen and ProtParam servers were used respectively. I-TASSER and VADAR servers were also used to model and refine the third structure of the studied structure, and the best model provided was refined using the GalaxyRefine server, and its analysis was also done through the VADAR server. Finally, the best third structure model was used for protein-protein docking studies.

#### RESULTS AND DISCUSSION

According to the obtained results, the molecular weight of the recombinant structure designed in this research is 45.172 kDa. The analysis of the antigenicity index of this construct was determined to be 0.7184 based on the Vaxigene server report, so it can be considered an antigenic construct. Molecular docking results showed that HBHA domain can bind to TLR4/MD2 receptor with abundant hydrogen bonds.

#### CONCLUSION

The results of this research indicated that the resulting structure has the ability to be used as a candidate in the production of recombinant vaccines against *Brucella millitensis* bacteria.

**Keywords:** Brucellosis, recombinant vaccine, bioinformatics, Omp25- HBHA

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Distribution of *aac(6')/aph(2'')*, *ant(6')*, *aph(3')-IIIa*, and *ant(4')-Ia* genes *Staphylococcus aureus* strains collected from Cockroaches obtained from hospitals environment in North of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Recently, research has demonstrated the importance of insect-borne illness transmission to humans. Cockroaches are among these insects that can pose a threat to human health due to their feeding habits and natural habitat in sewage and other environments. These insects are significant in the field of medicine, as they often carry approximately 40 different species of harmful bacteria that can infect vertebrates. *Staphylococcus aureus* is one of the most significant germs that can be spread by cockroaches. The aim of the current study was to investigate the aminoglycoside-modifying enzymes genes (AME) among *S. aureus* strains that were isolated from cockroaches in hospitals environments affiliated with Babol University of Medical Sciences.

### MATERIALS AND METHODS

Fifty strains of *Periplaneta americana* and *Blattella germanica* cockroaches were gathered, of which 25 were *P. americana* cockroaches (50%) and 25 were *B. germanica* cockroaches (50%). The internal and external surfaces of the cockroaches were subjected to bacterial isolation. The samples were assayed to detect the presence of three AME genes by the use of a triplex polymerase chain reaction (PCR) method.

### RESULTS AND DISCUSSION

A total of 50 cockroaches were collected; out of these, 10 (20%) *S. aureus* strains were isolated. Among the infected cockroaches, 70% were from *P. americana*, while 30% were from *B. germanica*. Furthermore, three strains were isolated from the inner surface of *B. germanica*, whereas six strains were isolated from the inner surface of *P. americana*, and one strain was isolated from the outer surface. Based on the PCR results, the frequencies of the genes *aac(6)-aph(2)*, *ant(6)-Ia*, *aph(3)-IIIa*, and *ant(4)-Ia* were 10%, 10%, 0%, and 0%, respectively.

### CONCLUSION

The *aac(6)-aph(2)* and *ant(6)-Ia* were the most frequent gene encoding resistance to gentamicin and other aminoglycosides. Consequently, hospital cockroaches are considered as a potential mechanical vector for *S. aureus* strains. Hence effective preventive and control measures are required to minimize cockroach related infections.

**Keywords:** *Staphylococcus aureus*, Hospital Cockroaches, aminoglycoside-modifying enzymes genes

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Cloning and expression of the HBHA-Omp25 recombinant sequence in a prokaryotic expression system

Maryam Farrukhpour <sup>1</sup>, Amin Jayderi <sup>2\*</sup>, Ali Foroohar Mehr <sup>3</sup>, Nemat Shams <sup>4</sup>, Narges Nazifi <sup>5</sup>

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Brucellosis (malt fever disease) is one of the most important diseases that is transmitted between humans and animals, this disease is of special interest in Iran in terms of both economic and public health aspects. The use of recombinant vaccines is one of the newest ways to deal with the side effects of common vaccines and to improve immunity. Therefore, in this study, the design and preparation of HBHA-Omp25 immunological conjugated construct consisting of Omp25 extramembrane antigen and HBHA molecular adjuvant and the feasibility of expressing this construct in prokaryotic expression system are investigated

### MATERIALS AND METHODS

In the present study, after HBHA and Omp25 gene fragments were extracted, they were amplified through PCR reaction. Also, SOE-PCR reaction was used in order to perform chimera and connect HBHA and Omp25 gene fragments. Enzymatic digestion of HBHA-Omp25 recombinant chimera fragment and pET22b (+) vector was performed through NCOI and ECORI cutting enzymes. It should be noted that HBHA-Omp25 fragment was placed inside the vector during the ligation process. In order to multiply the recombinant vector containing the HBHA-Omp25 chimeric fragment, the said fragment was transferred into the susceptible *E.coli* (DH5 $\alpha$ ) cell using the heat shock process, and then after extracting the plasmid, the recombinant plasmid was again transferred into the *E.coli* BL21 (DE3) susceptible cell. In addition, induction of gene expression was also done using 1 mM IPTG. Finally, the produced protein was purified using a nickel column and visualized. Also, gene expression was evaluated using SDS-PAGE gel.

### RESULTS AND DISCUSSION

According to the results obtained from the PCR, SOE-PCR and Colony-PCR reactions, the amplification of gene fragments, chimeras and the process of transferring the recombinant vector in susceptible DH5 $\alpha$  and BL21 (DE3) cells through heat shock were successful. Finally, the obtained results showed that the expression and purification of the recombinant protein has been done successfully.

### CONCLUSION

The results of this research were that the production of HBHA-Omp25 recombinant protein was successfully carried out. And the resulting structure has the ability to be used as a candidate for making recombinant vaccines against *Brucella melitensis* bacteria.

**Keywords:** Brucellosis, recombinant vaccine, prokaryotic expression system, Omp25- HBHA

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Bacterial typing using CRISPR loci; a feasibility assay

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#### ABSTRACT

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas systems are an adaptive, bacterial defense system against invading DNA (and RNA in some cases) such as phages and plasmids. CRISPR arrays comprise short, almost unique sequences called spacers that are interspersed with conserved palindromic repeats which can be counted in different bacterial isolates. CRISPR system is also used in genetic engineering and detection of pathogenic agents. In addition, these unique regions are diverse in number and sequence composition in different pathogenic bacteria and thereby can be a suitable candidate for molecular epidemiology and genotyping studies.

Emerging CRISPR-based typing methods open new avenues for high-resolution typing of a broad range of bacteria and constitute a practical means for rapid tracking of a diversity of food-associated microbes. Several studies have provided proof of concept in health-promoting genera (and species) such as *Lactobacillus* (e.g., *Lactobacillus casei*) and *Bifidobacterium* (e.g., *Bifidobacterium animalis* subsp. *lactis*), as well as starter cultures (e.g., *S. thermophilus*), food spoilage organisms (e.g., *L. buchneri*), and a plethora of human pathogens that pose a food safety threat (i.e., *Salmonella*).

CRISPR typing is a new molecular subtyping method to track the sources of pathogenic bacterial outbreaks and shows a promise in typing *Cronobacter*, however, this molecular typing procedure using routine PCR method has not been established. Therefore, the purpose of this study was to establish such methodology, 257 isolates of *Cronobacter sakazakii*, *C. malonaticus*, and *C. dublinensis* were used to verify the feasibility of the method. Results showed that 161 *C. sakazakii* strains could be divided into 129 CRISPR types (CTs). Compared to multi-locus sequence typing (MLST), this new molecular method has greater power to distinguish similar strains and had better agreement with whole genome sequence typing (WGST). With these explanations, it has been widely used in bacterial typing due to sensitivity, specificity and cost effectiveness.

**Keywords:** Bacterial typing, CRISPR loci, CRISPR-Cas, Genotyping

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Laboratory Model to Investigate the Effect of Cold Plasma in Cleaning Fungal Contamination from the Surface of Stone Cultural Heritage

Rezvav Ghaedi, Mohamad Mortazavi, Hamidreza Ghomi Marzdashti, Parisa Mohammadi, Atefe Shekofte

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### Abstract

Microorganisms are considered as one of the most important factors in the deterioration of tangible cultural heritage, which can cause irreversible damage to historical monuments and artefacts, and lead to the loss of their artistic, aesthetic values. Therefore, cleaning and removing living organism by different methods is one of the conservation treatments, which are carried out in the field of restoration and preservation of historic and cultural objects. It is very important to choose the cleaning method and the strategy to deal with microorganisms according to the nature and condition of the historical monument. The efficiency of new methods of restoration and cleaning have been less the subject of restoration and conservation research in the country. The purpose of this study is to adopt a suitable method to evaluate the efficiency of cleaning of stone heritage contaminated with *Aspergillus niger* fungus using atmospheric cold plasma in a laboratory model.

According to theoretical principles and ethical considerations concerning the conservation and restoration of cultural heritage, a laboratory model was designed for this research. Hence, carbonate stone similar to historical sample was obtained from Pasargadae region. Stone specimens were contaminated with *Aspergillus niger* strain MJ, which was stored in the microbiology bank of Alzahra University, used for inoculation on stone samples. After inoculation, stone samples were incubated for 6 months. Some stone specimens were considered as controls and three non-thermal plasma regimes, including dielectric barrier discharge, gliding arc and jet were used to clean *Aspergillus niger* from stone.

In order to investigate the effectiveness of the plasma-cleaning methods and whether they meet the needs of conservation and restoration area, an approach integrating complementary techniques were used, including colorimetry, optical microscopy, scanning electron microscopy and x-ray diffraction. Color changes, morphological and compositional features of the surface of samples were studied before and after inoculation by *Aspergillus niger*, and also after cleaning. The results showed that the fungus has grown well on the carbonate stone and in some cases the presence of halite and oxalate caused by the metabolite of the fungus was observed. The obtained results showed the reduction of the microbial load of this type of fungus by using three plasma regimens, although the structure of stone was changed. Accordingly, laboratory modeling of cleaning methods is necessary before any conservation treatment to less the damages before any implementation. More research should be performed on the introduction of proper laboratory methods to evaluate the cleaning techniques for different substrates.

**Keywords:** Carbonate stone, Deterioration, Fungi, Biomineralization, Decontamination, Cold plasma.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of bacterial infection and antimicrobial resistance patterns in the sputum of inpatients and outpatients during the COVID-19 pandemic

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Hospital-acquired infections are one of the concerns of medical centers. The risk of bacterial infections in hospital intensive care units has increased with the spread of the coronavirus. The aim of this study is to investigate the prevalence of bacterial infections and evaluate antibiotic resistance in sputum samples obtained from hospitalized and outpatient individuals during the COVID-19 pandemic.

### MATERIALS AND METHODS

The identification of bacteria in sputum samples was carried out through gram staining, specific culture media (EMB and blood agar), and biochemical tests on patients admitted to Kosar and Sina Hospital in Semnan, Iran, from September 2020 to September 2022. A comprehensive investigation was conducted to assess the susceptibility of the bacteria to 37 different antibiotics.

### RESULTS AND DISCUSSION

Among the 100 admitted patients, 51% were female. The mean age of the patients was 51.5 years. Out of the 49 patients diagnosed with bacterial infection, 38 were specifically admitted to the intensive care unit and coronary care unit. Furthermore, 54% of the patients in the study were found to be infected with COVID-19. Among the identified infections, 75.5% were caused by gram-negative bacteria. A total of 10 different bacteria were identified, with *Acinetobacter* (24.4%) and *Klebsiella* (22.4%) being the most prevalent, while *Citrobacter* and *Escherichia coli* (2%) were found to be the least common.

TE, CP, SXT, GM, FOX, CRO, MEN and FEP antibiotics were the most commonly used and the resistance rate was 40-50%. The highest levels of antibiotic resistance were observed in *Pseudomonas* 86% (TE>SXT/TIC>V/CC/FM/SAM/FOX/CZ), *Enterobacter* 80% (CZ>V/CC/SAM/FOX> V/OX/RA/S/AM), *Acinetobacter* 69% (CZ>CRO >MEN/FEP/FOX> CP/TE>SXT), and *Klebsiella* 36% (SAM>CZ> FOX/CRO/SXT> MEN/FEP/CP) when gram-negative bacteria were co-infected with coronavirus. Also, when infected with gram-positive bacteria and coronavirus, the highest levels of antibiotic resistance were found in *S. epidermidis* 75% (OX>AZM>TE/CP/CC/CM/SXT) and *Streptococcus* 60% (SXT>S>CP). No statistically significant association was found between co-infection with bacterial infections and COVID-19.

### CONCLUSION

Patients who were admitted to intensive care units with COVID-19 displayed increased vulnerability to healthcare-associated infections caused by antibiotic-resistant bacteria. Insufficient and prompt medical care in patients who experienced co-infection with pathogenic microorganisms resulted in fatal outcomes, exacerbating the situation due to the presence of antibiotic resistance in these microorganisms.

**Keywords:** COVID-19, Bacterial infection, Antibiotic resistance, Sputum

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Cloning, expression and purification of papain enzyme in *E. coli* host

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Papain is a plant proteolytic enzyme that belongs to the cysteine protease family. It is found naturally in papaya and is manufactured from the latex of raw papaya fruits. Papain is able to break down organic molecules made of amino acids, known as polypeptides, and thus plays a crucial role in diverse biological processes in physiological and pathological states, drug designs, industrial uses such as meat tenderizers, and pharmaceutical preparations. Extraction and purification of papain from natural sources is costly and inefficient. Therefore, this study focuses on the cloning, expression, and purification of the papain enzyme in a bacterial host.

#### MATERIALS AND METHODS

An optimized sequence of papain gene was created according to the submitted genes in the NCBI along with a His-tag, in pET21a expression vector by the use of GeneRunner software and Genescript online application. Then the sequence was placed in the ordered vector by a Chinese company.

The cloned papain gene was introduced into *Escherichia coli* BL21(DE3) as the expression host via CaCl<sub>2</sub> method of competent cell preparation, and the target protein was expressed by IPTG as the inducer.

Expression optimization was done in different conditions (IPTG concentration (0-1 mM final concentration) and growth temperature (20, 28 and 37 °C)). The produced protein was purified using Ni-NTA column chromatography. SDS-PAGE analysis was performed to evaluate the expression of cloned gene and to follow the purification process. Enzyme activity was monitored via treatment of Skim milk agar (1% W/V) by different chromatography samples.

#### RESULTS AND DISCUSSION

The transformed bacteria could easily grow on LB Agar containing Ampicillin (beta lactamase as resistant gene of vector). The most suitable concentration of IPTG at 20°C was 0.1 mM, it may be related to the antibacterial activity of papain. During purification with Ni-NTA chromatography and SDS-Page analysis the desired molecules of enzyme emerged in SDS-PAGE and could show protease activity on Skim milk agar.

#### CONCLUSION

Overall, this study demonstrates the successful cloning, expression, and purification of papain enzyme in a bacterial host. Further analysis for optimization of papain purification is now in process in our research group.

**Keywords:** papain, His-tag, chromatography, SDS-PAGE

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation and identification of effective bacteriophages against *Enterococcus faecalis* bacteria from hospital wastewater

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Enterococcus* is a family of gram-positive, non-spore-forming bacteria consisting of 38 species. One of the most important of this family is *Enterococcus faecalis*, which lives in the human intestine. *E. faecalis* as a member of the mammalian gastrointestinal flora, is a major cause of nosocomial infections and a growing public health problem. Phage therapy is a viable alternative to antibiotics for treating microbial infections, particularly managing drug-resistant strains of bacteria. The aim of this study was to isolate and identify effective bacteriophages against *E. faecalis* isolated from hospitalized patients.

### MATERIALS AND METHODS

Fifty-eight *E. faecalis* were isolated from urine, wound, abscess and blood samples from patients admitted to Resalat Hospital in Tehran, Iran, and identified by biochemical and molecular methods. The phage was isolated from hospital wastewater using the double layer agar method and was characterized using transmission electron microscopy (TEM). Phage host range was determined using spot test. The stability of isolated phage was tested at acidic and alkaline pH, at high temperatures, and in cold storage. Phage efficacy (MOI) was also investigated with regard to the bacterium/phage ratio. The effect of isolated bacteriophages against *E. faecalis* One, Three and five day old preformed biofilm was investigated using the microtitre plate method.

### RESULTS AND DISCUSSION

According to Electron microscopy observations, the isolated phage belonged to the *Siphoviridae* family. Spot testing on the collected isolates indicated that bacteriophage was able to lyse 40 out of 58 isolates (68%) of *E. faecalis*. Suitable pH spectra for phage survival was 6–10, at which the phage showed 100% activity. The optimal temperature for phage growth was 30–45°C, with the highest growth at 37°C. The results revealed that this isolated phage had low salt tolerance. The optimal MOI for isolated phage was 0,01. The results showed a 10-100-fold decrease in viable cells (CFU/biofilm) after phage treatment.

### CONCLUSION

The characterization of bacteriophages with a diverse host range of *E. faecalis* could help in the development of effective therapeutic strategies for this pathogen.

**Keywords:** Bacteriophage; *Enterococcus faecalis*; Phage therapy

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Novel applications of viruses in noncoding RNA therapy for gastrointestinal cancers

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Respecting the high mortality rate of gastrointestinal (GI) cancers, there is an urgent need to propose novel therapeutic approaches to overcome GI cancers. Utilization of viruses in regard to realign ncRNA transcription to cease tumorigenesis in addition to their capability of coding siRNAs and miRNAs to silence oncogenes highlights their inimitable competence as anti-tumor vectors. Moreover, intrinsic features of viruses as drastic immunogens to induce immune response along with unsurpassed ability of oncolytic viruses to target specific tumors represents them as potent anti-cancer vectors. In this review, we will discuss novel applications of viruses in ncRNA based GI cancers therapy as a treatment method capable of rectifying deficiencies of traditional treatments.

#### MATERIALS AND METHODS

To conduct this research, we studied articles regarding ncRNA therapy, gastrointestinal cancers and viral vectors on PubMed and Google Scholar.

#### RESULTS AND DISCUSSION

Implementation of viruses in ncRNA therapy is a multilateral combat strategy against GI cancers. Besides their ability in coding ncRNAs, direct lysis of virus-infected tumor cells leads to the release of tumor antigens, altering innate immune system as well as leaving the tumor microenvironment exposed. Utilization of ncRNA therapy in combination with immunotherapy is facilitated thanks to viral vectors, as monoclonal antibodies can be fused to viral capsids. Adenoviruses are outstanding viral vectors utilized in ncRNA therapy as they are capable of coding siRNAs to silence PI3K gene, which results in apoptosis of gastric cancer (GC) cells and inhibits proliferation. Lentiviruses are RNA viruses inducing down-regulation of synuclein with RNA interference (RNAi) as well as a decline in tau phosphorylation with siRNAs. Furthermore, Semliki Forest virus (SFV) is an alphavirus with miRNAs capable of tumor targeting and tumor growth inhibition.

#### CONCLUSION

Application of viruses in ncRNA Therapy has exhibited promising results. However, low efficiency of viral vectors in tumor mass penetration, antiviral immune response of host and instability of ncRNAs should be addressed. Nevertheless, ncRNA therapy and oncolytic viruses will soon become an integral part of GI cancers therapy.

**Keywords:** gastrointestinal cancers, ncRNA therapy, viral vectors, gene therapy, oncolytic viruses

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Design and synthesis of chimeric antimicrobial peptide and transfer it to Escherichia coli strain B121

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The Unlimited use of antibiotics nowadays has led to many problems, including the appearance of antibiotic-resistant bacteria. For this reason, the use of antimicrobial peptides can be a good alternative to antibiotics. The aim of this research is to design and manufacture a chimeric antimicrobial peptide and transfer it to a suitable vector.

### MATERIALS AND METHODS

At first, a search was made in scientific databases to find antimicrobial peptides. Next, the appropriate peptide was selected using the docking process. The nucleotide sequence of the selected peptide was optimized for better performance and compatibility in the direction of production in E. coli. The selected nucleotide sequence was inserted into pET26 vector. After the plasmid containing this sequence was prepared, the stages of preparation and growth of the host bacteria were carried out. Then transformation processes were carried out into the bacterial host using heat shock.

### RESULTS AND DISCUSSION

The growth of bacteria in the positive transfer samples and the lack of growth of bacteria in the negative transfer samples indicate that the plasmid was received by the expression vector, and the PCR process was also done to ensure that. The production of this recombinant peptide was also done under optimal induction conditions.

### CONCLUSION

Optimizing the expression conditions for chimeric antimicrobial peptide production was done using pET26 vector by host Escherichia coli strain B121. which can be used for therapeutic purposes to combat antibiotic-resistant bacteria.

**Keywords:** docking, antimicrobial peptide, antibiotic resistant bacteria, gene transfer

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular Characterization of $\beta$ -lactamase-producing multidrug-resistant Uropathogenic *Escherichia coli* isolated from urinary tract infections in southwest of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Infections with  $\beta$ -lactamase-producing multidrug-resistant *E. coli* is associated with poor outcomes due delays in initiation appropriate antimicrobial therapy and limited therapeutic options, leading to increase in social cost such as prolonged hospitalization and increased medical expenses. This study was aimed to characterized antimicrobial susceptibility patterns, ESBL phenotype, acquisition of  $\beta$ -lactamase genes, ERIC-PCR profile of  $\beta$ -lactamase-producing multidrug-resistant Uropathogenic *Escherichia coli* strains.

### MATERIALS AND METHODS

The cross-sectional study was conducted at Ahvaz Jundishapur University of Medical Sciences, Iran, between November 2015 and March 2016. Antimicrobial susceptibility tests were performed by disk diffusion method according to the CLSI guidelines and then, the ESBL production was phenotypically determined by both double disk synergy test and combined disk test. All multidrug-resistant strains of *E. coli* isolates were subjected to molecular analysis.

### RESULTS AND DISCUSSION

From total 107 samples defined as ESBL positive by using drug susceptibility testing, 55 MDR isolates were subjected to detection of the common ESBL genes and ERIC-PCR analysis. However, considerable resistance was observed to ampicillin (98.1%), piperacillin (96.4%) azithromycin (83.6%), cephalosporins (85- 92.5%) and SXT (80%). The presence of *CTX-M* gene was the most pronounced, followed *TEM* gene, then *SHV* genes. By ERIC-PCR, the high heterogeneity was observed among the isolates from UTIs including 35 isolates which were categorized in 17 clusters and 20 single clones.

### CONCLUSION

Due to the high level of genotypic heterogeneity in MDR strains in the present study and the simultaneous presence of resistance extended spectrum beta-lactamases (ESBL) genes, it can be a warning danger to the health community that should be avoid to dispersal as soon as possible to prevent the further spread of these strains.

**Keywords:** Uropathogenic *Escherichia coli*, ESBL, MDR, PCR, Genotyping Techniques.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Tracking Antibiotic Resistance Trends in Central Iran Amidst the Covid-19 Pandemic from 2021 to 2023: A Comprehensive Epidemiological Study

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

The emergence of the coronavirus disease 2019 appears to have an impact on antibiotic resistance patterns. It is possible that specific circumstances during the Covid-19 era could be influential in the escalation of antimicrobial resistance (AMR). This study aimed to examine the alterations in antimicrobial resistance patterns of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* in Al-Zahra hospital.

#### MATERIALS AND METHODS

Over a span of three years, from March 2021 to January 2023, a total of 3651 clinical samples were collected from patients who were hospitalized at Al-Zahra Hospital in Isfahan. The standard methods the Clinical and Laboratory Standards Institute (CLSI) recommended for identifying gram-negative bacteria and conducting antibiotic susceptibility testing were employed. We categorized the data into three years.

#### RESULTS AND DISCUSSION

Highest resistance rates were seen in *Acinetobacter baumannii* to Ciprofloxacin (98.0%) and Ampicillin-Sulbactam (97.0%). For *P. aeruginosa* the resistance rate for ceftazidime (36.1), Levofloxacin (37.8) and Meropenem (47.1) dropped seriously in 2022.

#### CONCLUSION

During the second year of the pandemic in central Iran, all three species evaluated in this study have exhibited increasing rates of antimicrobial resistance (AMR). This can be attributed to two peaks that occurred on May 6th, 2021, and August 27th, 2021, within Iran. The findings of this study indicate that *P. aeruginosa*, *K. pneumoniae*, and *A. baumannii* strains in central Iran possess a higher degree of antibiotic resistance compared to previous studies conducted prior to the pandemic.

**Keywords:** Antibiotic resistance, COVID-19 pandemic, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# An In silico analysis to study the molecular host-microbe in tuberculosis infection: disturb the immune system by interacting with CXCR8 and TLR2

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Tuberculosis is a bacterial infectious disease caused by *Mycobacterium tuberculosis* (Mtb), which by 10.4 million new cases and nearly 1.7 million deaths in 2017, causing more deaths worldwide than any other infectious agent. Mtb adopt diverse strategies to survive and invade human immunity. These mechanisms make the pathogen resistant to presently available drugs, a primary contributing factor in the failure to control the spread of tuberculosis. It has been shown that interacting bacterial proteins with human proteins can change signaling, metabolic pathways, and cellular processes in the infected host. This study aimed to use bioinformatics tools to understand how Mtb proteins develop infection in human cells.

### MATERIALS AND METHODS

To identify bacterial proteins that interact with human proteins, ImitateDB database was used. The Mtb (strain ATCC 25618 / H37Rv) was selected in this database, and interacting proteins with human cells were recognized. Host interactor protein IDs were extracted as well. These codes were converted into gene symbols through the BioDBnet database, and these proteins' functions were determined using string and EnrichR databases and string.

### RESULTS AND DISCUSSION

The ImitateDB showed Mtb proteins including serA (D-3-phosphoglycerate dehydrogenase), ahcY (Adenosylhomocysteinase), atsG (arylsulphatase), glmU (N-acetylglucosamine-1-phosphate uridyl transferase) mainly interact with CXCR8 (Chemokine (C-X-C motif) receptor 8) protein while PE35 and PPE68 bind to TLR2 (Toll-like receptors 2) protein in human cells. As presented in Figure 1, these two proteins are also related to 10 human proteins through different motifs. Functional analysis by EnrichR revealed that all these human genes are significantly involved in diseases associated with Toll-like signaling and MyD88 (Myeloid differentiation primary response 88) protein deficiency. (Figure 2). Various studies have shown that disruptions of TLR2 and MyD88 signaling pathways interfere with other intrinsic intracellular antimicrobial pathways, such as autophagy and functional vitamin D receptor (VDR) signaling, in addition to causing acute inflammation and lung injury.

### CONCLUSION

Bioinformatics approaches can be used for understanding of the molecular mechanisms of tuberculosis pathogenesis and host protective immunity against tuberculosis infections, and to facilitate vaccine design and development.

**Keywords:** *Mycobacterium tuberculosis*, Bioinformatics analysis, protein-protein interaction, molecular mechanism

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Decolorization of some reactive azo dyes using bacteria isolated from the wastewater treatment systems

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The presence of azo dyes in the environment is an issue of major concern since they are highly recalcitrant and toxic. Azo dyes are extensively used in textile industries, and account for more than 50% of the synthetic dyes used worldwide. Releasing azo dyes into water bodies results in aesthetic problems and deteriorates water quality. The aim of this research was investigation on removal reactive azo dyes using biological treatment.

### MATERIALS AND METHODS

To isolate some bacteria able to decolorize two commercial reactive dyes including Reactive Orange 122 (RO122) and Reactive Red 195 (RR195), wastewater samples were collected from activated sludges. The samples were inoculated into R2A medium containing 50 mg L<sup>-1</sup> of individual dyes and incubated at 28 °C under static conditions for 10 days. The growth culture was transferred into fresh medium every 7 days and decolorization were examined every day using spectrophotometer.

### RESULTS AND DISCUSSION

Results were demonstrated that the microbial consortium was able to decolorized both RO122 and RR195 dyes due to decreasing the optical density at 495 and 545 nm, respectively. The rate of microbial decolorization of RO122 and RR195 was 81% and 56% after 10 days, respectively. These results shown that the microbial consortium isolated from dye-contaminated environments can decolorize azo dyes. The decolorization rate of the azo dyes depended on the chemical structure and the position of functional groups proximal to the azo bond. Both azo dyes used in this study were reactive and contain one azo group.

### CONCLUSION

The results revealed that the microbial consortium was successfully decolorized both RO122 and RR195. Microbial decolorization technique are very useful to remove textile dyes from environments.

**Keywords:** Bacterial decolorization, azo reactive dyes, wastewater

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibacterial evaluation of some Iranian honeys: whole honeys vs. their characterized phenolic extract

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

In recent years, the pharmaceutical interest in honey due to its antimicrobial properties against antibiotic resistant bacteria has increased. The aim of this study was to assess and compare the antimicrobial activity of whole honeys and their phenolic extract on clinical strains of *Escherichia coli* and *Staphylococcus aureus*.

### MATERIALS AND METHODS

In present study, phenolic compounds were isolated from 25 Iranian honeys of different botanical and geographical origins by solid-phase extraction (SPE). Characterization of honey phenolic extracts was carried out by HPLC-DAD-ESI-QTOF/MS. Disc diffusion method was used for determination of antimicrobial properties of whole honeys and their phenolic extract.

### RESULTS AND DISCUSSION

In this study, the majority of extracts exhibited the inhibition zone lower than whole honey, but phenolic fraction of some Iranian honeys (samples from White clover, Hawthorn, Black cumin, and Jujube botanical sources) exerted better antimicrobial activity than whole honey. Characterizing phenolic compounds, we observed the major amounts of methyl syringate and phenyllactic acid in Hawthorn honey, while chrysin and pinocembrin were present in great quantities in White clover and also Hawthorn honeys.

### CONCLUSION

In conclusion, four phenolic extracts indicated lower zone of inhibition than the whole honey pointing out that honey phenolic fraction may exert antimicrobial activity and contain a source of antimicrobial compounds to develop functional ingredients.

**Keywords:** Iranian honeys, antimicrobial activity, phenolic extract

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Control of Infection, Antibiotic Resistance and days of future.

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#### ABSTRACT

#### BACKGROUND-AND-OBJECTIVES

Antibiotic resistance is currently one of the main global health challenges. To prevent the flood of deaths due to antibiotic resistance among patients, it is reasonable to prevent this disaster in the first place and for this achievement need to a cooperation between the patient, doctors, nurse and other medical-staff.

#### MATERIALS-AND-METHODS:

In this study by closely observing the patients' problems, several ways to reduce infection and antibiotic resistance are suggested.

#### RESULTS-AND-DISCUSSION

1: Doctors should avoid prescribing antibiotics as much as possible. For this purpose, it is suggested that a unit be established in the hospital for explaining alternative methods and awareness and education about the prudent use of antibiotics to patients.

2: The correct division of patients in the hospital. For example, a patient with a fungal disease should not be admitted close to a diabetic patient with an infected wound, because fungal spores may spread through the air and make the diabetic patient's wound worse.

3: Separating patients who have a high risk of infection from others and preventing people from meeting the patient (meet the patient from behind the glass).

4: Isolation of patients who have diseases that are transmitted through the air (such as measles) in special and closed rooms.

5: It is recommended that two nurses go to the patient's bed at the same time during any dressing change or drug injection or any sampling and touch or contact with the patient.

6: Cleaning and disinfection of the room and medical fluid, devices and air (suggested to give certain hours a day for this task and even take the patient to another room temporarily and sterilize the entire space of the room with UV-light.)

7: If the drug dose is prescribed incorrectly or the course of antibiotic use is completed incompletely, it is possible to enrich the resistant subspecies of bacteria and thus cause an increase in resistance.

#### CONCLUSION

Considering that Iran ranks second in antibiotic consumption in the world and the increasing prevalence of antibiotic resistance, it is time to act, not talk. I suggest that basic measures should be taken like the few suggestions mentioned above.

**Keywords:** Infection, Antibiotic-Resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Distribution of minor virulence genes among *Clostridium perfringens* Isolates

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#### ABSTRACT

*Clostridium perfringens* (*C. perfringens*) belongs to the family of *Clostridiaceae* and produces a wide range of toxins (four major and a variety of minor toxins). Some toxins associated with virulence have been shown to participate in the pathogenesis of enteric diseases in sheep, goats, and other animals. The aim of this study was to determine the presence of some minor virulence genes and their genetic diversity in *C. perfringens* isolates.

About 84 isolates collected from sheep and goat flocks were provided by the microbial archive of Razi Institute (south-east branch) that located in Kerman. Isolates were removed from the cryotube stored at  $-70^{\circ}\text{C}$ ; then, it smeared on blood agar medium containing 5 % defibrinated sheep blood, which was incubated in an anaerobic condition. After DNA extraction, detection of toxin genes (*cpb2*, *tpel*, and *PFO*) was carried out, using three pairs of specific primers were examined and sequenced for the presence of minor virulence genes (*PFO*, *cpb2* and *tpel*) by PCR method. Purified PCR products were sequenced using the Sinacolon facility, Tehran, Iran. The gene sequences were aligned according to their nucleotides, using computer program MEGA 7. Then, analysis was performed for confirm the nucleotide sequences, Results showed that *PFO* and *cpb2* were found in 79 out of 84 (94.4 %) and the presence of *tpel* was confirmed in 29 out of 84 (35 %) isolates so the dominant minor virulence genes were *PFO* and *cpb2*. prevalence of these genes in *C. perfringens* isolates would provide more information regarding the importance of these toxins and lead to a greater understanding of the pathogenesis of diseases caused by *C. perfringens*, furthermore DNA sequencing revealed closed relationships with others world strains that were range approximately (97–100 %) with the GenBank database.

**Keywords:** *C. perfringens*, minor virulence genes, Sequence, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The biological activities of phytosterol extracted from pistachio

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Phytosterols (PSs) are plant-originated steroids. Health-promoting effects of PSs include anti-obesity, anti-diabetic, anti-microbial, anti-inflammatory, antioxidant, and immunomodulatory effects. Other studies showed that the ethanolic and hydroalcoholic extracts of different parts (e.g., leaf and fruit) of *Pistacia vera* L. had antioxidant properties. The consumption of nuts, including pistachio, seems to be useful in improving the intestinal microbial composition. The evaluation of critical sterols such as campesterol,  $\beta$ -sitosterol, stigmasterol, epicoprostanol, etc. against a wide range of bacteria revealed notable antibacterial properties. This is the first report on the antioxidant and antibacterial activities of phytosterol extracted from the green skin of three variants of Damghan's pistachio.

#### MATERIALS AND METHODS

The Soxhlet method was used to extract total phytosterol from green pistachio skin of Akbari, Khanjari, and Abbasali variants of Damghan's pistachio, which was obtained from Damghan gardens. The antioxidant activity of the extracted phytosterols was examined using the FRAP method with three repetitions and five different concentrations. Ascorbic acid was used as the control solution. The antibacterial properties of the extracted phytosterols were evaluated against *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) bacteria in duplicate. Statistical analysis of the results of antioxidant activity was done using SPSS software (version 27).

#### RESULTS AND DISCUSSION

The antioxidant properties of the phytosterols extracted from three pistachios' skin types were high without any significant difference between them. The highest absorption was observed in concentration of 20 mg/ml in the studied samples. However, the extracted phytosterols showed no antibacterial activities at concentrations of 1.6-10000 mg/ml.

#### CONCLUSION

According to the present outcomes, the phytosterol extracted from the green skin of the studied Damghan's pistachios had good antioxidant properties. Accordingly, it is worth further studies on the application of phytosterol extracted from the pistachio's green skin in the pharmaceutical, food, and cosmetic industries. However, contrary to other reports, no antibacterial effect was seen for the extracted phytosterols. We suggest further investigation to find the reason for the seen discrepancy.

**Keywords:** Phytosterol, *Pistacia vera* L., antioxidant, antibacterial, Damghan

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Solvent extraction of *Lactobacillus plantarum* supernatant compounds and investigation of its antibacterial effects

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Interest in probiotics and probiotic-based functional foods has grown enormously during the last few years. The internationally endorsed definition of probiotics is live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Lactic acid bacteria (LAB) as Gram-positive, catalase-negative, and non-spore forming bacteria are the major group of probiotic technologically suitable microorganisms. This study was designed to determine the most potent antibacterial compounds from the solvent extraction of culture supernatant of a *L. plantarum* strain isolated from cheese.

### MATERIALS AND METHODS

Primarily, the cheese sample was prepared from East Azerbaijan province. Isolation of *Lactobacillus* bacteria and Sequencing were performed. A cell-free supernatant was prepared by 20 min centrifugation at 12000 rpm, 4°C, followed by passing through 0.22-micron filter. Sample was extracted successively with n-hexane and ethyl acetate (EtOAc) at room temperature. The extracts were concentrated under reduced pressure at 40°C using a Rotary evaporator instrument and kept at 4 °C to be further analyzed by *in vitro* antimicrobial assay. The MIC values of n-hexane and ethyl acetate extracts of the supernatant were determined against *Staphylococcus aureus* ATCC12600, *Escherichia coli* ATCC11775, *Shigella flexneri*, *Salmonella Typhi* PTCC1609, *Pseudomonas aeruginosa* ATCC27853, *Pseudomonas aeruginosa* ATCC1045 *Candida albicans* ATCC10321, *Streptococcus mutans* ATCC35668, *Propionibacterium* 98-6912 and *Bacillus subtilis* ATCC6051.

### RESULTS AND DISCUSSION

Sequencing results led to the identification of *Lactobacillus plantarum* as the selected LAB isolate. We identified four species from the cheese sample and submitted 16S RNA genome data to national center for biotechnology information (NCBI) gene bank. The named gene data are available via accession numbers: OR157959, OR157958, OR157957, OR145146. The antimicrobial assay resulted in identification of the EtOAc extract as the best sample against *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri*, *Salmonella Typhi*, *Pseudomonas aeruginosa* ATCC27853 and ATCC1045, *Bacillus subtilis*, *Propionibacterium*, *Streptococcus mutans*, *Candida albicans*, with MIC values of 16, 4, 8, 8, 4, 8, 4, 4, 8, 32 mg/ml, respectively.

### CONCLUSION

Ethyl acetate extract played a dominant role and exhibited significant activities with promising MIC values of 4 mg/mL against some assessed microbial strains. Further chemical analysis of this extract for determination of effective antibacterial compound (s) are now is underway in our institute.

**Keywords:** Probiotic, Solvent extraction, Antibacterial effects.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Considerable Avian-associated Bacterial Zoonoses

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

In recent years, there has been a growing concern regarding zoonoses related to birds. These zoonotic diseases can be transmitted to humans through direct contact with pet birds or industrial birds, as well as through their remains, such as feces, dried fecal material, oral secretions, carcasses, and bird-derived food products like meat and eggs. While zoonoses associated with domestic mammals have received more attention in terms of eradication and control, avian-associated zoonoses have relatively been overlooked due to the more distant evolutionary relationship between birds and humans. However, with the increase in human population and subsequent expansion of poultry breeding for food supply, the proximity of humans to birds has led to the spread of these zoonoses, causing significant damage to both the poultry industry and human health. Bacterial zoonoses are of particular concern, given the emergence of antimicrobial resistance and the restrictions on antibiotic use, coupled with the potential transmission of these diseases through meat and eggs.

#### MATERIALS AND METHODS

This research aims to explore and present several bacterial zoonotic diseases of significance to human public health that can be directly or indirectly transmitted from birds to humans. The data were collected from international scientific databases, including Google Scholar, Scopus, Pubmed, and Elsevier. Additionally, the Persian Scientific Information Database of the Academic Center for Education, Culture, and Research to complement the analysis were utilized.

#### RESULTS AND DISCUSSION

Some of the most important bacterial zoonotic diseases related to birds include Avian tuberculosis, salmonellosis, colibacillosis, mycoplasmosis, botulism, campylobacteriosis, yersiniosis, ornithosis (psittacosis), clostridiosis, listeriosis, pasteurellosis, staphylococcus, streptococcus, erysipelothrix, spirochetosis and vibriosis.

#### CONCLUSION

Considering the Frequency of bird-keeping as pets and the significant role of poultry meat and eggs as a primary food source worldwide, it is crucial to prioritize research and attention to avian and poultry diseases, especially those associated with bird-to-human transmission. By studying infectious agents, implementing effective control measures, and focusing on eradication strategies, we can significantly contribute to ensuring the sustainability of the poultry industry and promoting better health outcomes in human societies.

**Keywords: Avian, Bacterial Zoonoses, Human Health, Birds**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Phototherapy with psoralen plus ultraviolet-A (PUVA) as an Effective Intervention for Preventing Biofilm Formation in *Staphylococcus aureus* isolated from Patients' Wounds

**Hadis Sadri<sup>1</sup>, Mahshid Akhoondian<sup>1</sup>, Parastoo Ehsani<sup>1</sup>, Mohammad Pooya<sup>1\*</sup>**

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Staphylococcus aureus* is a major pathogen associated with wound infections, and the formation of biofilms by this bacterium poses significant challenges to successful treatment. Biofilms not only protect bacteria from antibiotics but also contribute to chronicity and recurrence of infections. Therefore, novel strategies are urgently required to prevent biofilm formation. This study aimed to investigate the potential of phototherapy with psoralen-ultraviolet A (PUVA) as a new treatment method for preventing biofilm formation in *S. aureus* samples isolated from patients' wounds.

### MATERIALS AND METHODS

Specimens were collected from patients diagnosed with chronic infected wounds. The isolates were phenotypically confirmed using standard microbiological techniques. Biofilm-forming ability was assessed using crystal violet staining and microtiter plate assays. PUVA treatment involved exposing the bacterial isolates to a combination of 8-methoxypsoralen (8-MOP) and UVA irradiation at specific wavelengths and doses. Control groups consisting of untreated bacteria and bacteria treated with either psoralen or UVA alone were included for comparison.

### RESULTS AND DISCUSSION

A total of 30 *S. aureus* isolates were collected. PUVA treatment significantly inhibited biofilm formation and bacterial growth ability in 28 out of 30 isolates when the UVA dose was 500 mj/cm<sup>2</sup> and the 8-MOP concentration was 75 µg/ml or more. However, when the UVA dose increased to 1000 mj/cm<sup>2</sup>, in all 30 isolates inhibition of biofilm formation started from the minimum concentration of 8-MOP (50 µg/ml). The inhibition pattern continued with higher doses of UVA (5000 mj/cm<sup>2</sup>), while the 8-MOP concentration was at its minimum level. The effect of 8-MOP alone or UVA alone at any doses was similar to no-PUVA group.

### CONCLUSION

It seems that PUVA treatment significantly reduce biofilm formation in a dose-dependent manner in *S. aureus*. As we were seeking for the lowest doses of both 8-MOP and UVA, the optimal conditions for inhibiting biofilm formation seems to be 75 µg/ml of 8-MOP and 500 mj/cm<sup>2</sup> UVA. Further research is needed to explore the mechanisms underlying the inhibitory effects of PUVA treatment and to evaluate its potential clinical applications in preventing biofilm formation and improving the management of *S. aureus* infections.

**Keywords:** PUVA, biofilm, *Staphylococcus aureus*, wound infection, psoralen

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluation of antibacterial potential of lytic bacteriophages against infectious caused by *Acinetobacter baumannii* in burn wounds

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

One of the main invasive bacteria found in wound and burn infections, which has become more resistant to antibiotics than other bacteria, is *Acinetobacter baumannii*.

#### MATERIALS AND METHODS

30 strains of *Acinetobacter baumannii* were isolated from the wounds of patients from the hospitals of East Azarbaijan province. For all strains, the antibiogram test was performed with 15 different antibiotics, and 26 strains with multiple antibiotic resistance were selected for bacteriophage isolation against. Bacteriophage was isolated against *Acinetobacter baumannii* isolates using agay by-layer method. Then, the host range of bacteriophages was determined and phages with the widest host range were selected for more characterization. One step growth curve was determined and the stability of bacteriophages was evaluated in the environmental conditions (pH, UV and temperature).

#### RESULTS AND DISCUSSION

Based on the obtained results, one of the isolated bacteriophages had lytic effect against 15 bacterial isolates. A cocktail of four isolated phages showed lytic effect on 23 of 30 isolates. Based on one step growth curves, latent period of the phages was evaluated 20-60 min. According to the stability experiments, the selected bacteriophages maintained their activity in UV exposure up to 10 minutes. They were stable at pH value of 6-10 and temperature 4-60 °C. Examination of these bacteriophages by electron microscopy (TEM) showed that these bacteriophages were belonged to the *Tectiviridae* and *Podoviridae* families.

#### CONCLUSION

This study showed that phage therapy can be an effective alternative to fight antibiotic-resistant infections caused by *Acinetobacter baumannii*.

**Keywords:** Bacteriophage, Phage Therapy, *Acinetobacter Baumannii*, Antibiotic Resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation of the antibiotic susceptibility pattern of carbapenem-resistant *Klebsiella pneumoniae* isolated from patients at Pars General Hospital, Tehran, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Given the spread of antibiotic resistance and the increasing infections caused by Gram-negative bacilli over the past few years, a study was conducted to examine the antibiotic susceptibility pattern in carbapenem-resistant *Klebsiella pneumoniae* from hospitalized and outpatient individuals visiting Pars General Hospital, Tehran, Iran.

### MATERIALS AND METHODS

In the conducted study, all patient culture results, sample types, admission types, patient ages, and patient genders from February 2022 to July 2023 were extracted and analyzed using SPSS software.

### RESULTS AND DISCUSSION

Approximately half of the carbapenem-resistant *Klebsiella pneumoniae* isolates in this study (51.1%) were from individuals aged 65 and older, and most cases (77.8%) were from outpatient attendees. The frequency of isolation based on clinical sample type was related to wounds (40%), urine (35.6%), sputum (15.6%), catheters (4.4%), bronchoalveolar lavage (2.2%), and ascetic fluid (2.2%). The highest antibiotic susceptibility was observed for tigecycline (87.5%), colistin and polymyxin B (85%), followed by doxycycline (65%) and tetracycline (64.1%). While susceptibility to other tested antibiotics (amikacin, cefixime, cephalixin, cefotaxime, ceftazidime, cefazoline, cefepime, ceftriaxone, ciprofloxacin, levofloxacin, norfloxacin, ertapenem, imipenem, meropenem, ampicillin, amoxicillin, gentamicin, tobramycin, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ampicillin-sulbactam, aztreonam, minocycline, nitrofurantoin, and chloramphenicol) ranged from zero to 20.5%. Our findings in this study indicate the highest antibiotic sensitivity in carbapenem-resistant *Klebsiella pneumoniae* to tigecycline, colistin, and polymyxin B, aligning with other studies conducted in this field.

### CONCLUSION

The results of these studies demonstrate high resistance incident of carbapenem-resistant *Klebsiella pneumoniae* to most available antibiotics, which is significant in the treatment of patients infected with this microorganism.

**Keywords:** Antibiotic Resistance, Carbapenem, *Klebsiella pneumoniae*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of antibiotic resistance pattern of uropathogenic *E. coli* isolates in children with urinary tract infection hospitalized in Tehran

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

Urinary tract infection (UTI) is common in children and *Escherichia coli* is its primary pathogen. Although difficult to diagnose in infants and young children, accurate diagnosis and appropriate antimicrobial treatment are crucial to prevent long-term consequences. However, traditional antibiotics may not be effective due to increasing rates of acquired resistance. This study aimed to examine the antibiotic resistance pattern in *E. coli* strains isolated from pediatric UTI patients.

### MATERIALS AND METHODS

Bacterial samples were isolated from the pediatric wards at Imam and Loghman hospitals in a period of 6 months. Of these, *E. coli* bacteria were confirmed using biochemical methods and PCR. Antibiotic sensitivity, multi-drug resistance (MDR) and extended spectrum beta-lactamase (ESBL) were determined using disc diffusion assay, following standard procedures recommended by CLSI.

### RESULTS AND DISCUSSION

Forty *E. coli* isolates (20 from each hospital) were recovered. The ratio of girls to boys was equal in Loghman Hospital and 3 to 1 in Imam Hospital. Most of them were  $\leq 3$  years old. The rate of antibiotic resistance was high in both hospitals while there was no resistance among the isolates to nitrofurantoin. In general, the isolates from Imam Hospital were more resistant than those from Loghman Hospital. Therefore, the isolates that resistant to a large number of antibiotics ( $\geq 8$ ) were mainly found at Imam Hospital, while those resistant to fewer antibiotics ( $\leq 7$ ) were mostly recovered from Loghman Hospital. No isolate in Loghman Hospital was resistant to imipenem, in comparison to 60% resistance in Imam Hospital's isolates. Among the isolates, 95% were MDR, with over a third (37%) being highly resistant to 10 or more antibiotics, while the rest were resistant to 3 to 9 antibiotics (regular MDR). Again, the ratio of the highly resistant isolates was 2.5 times higher at Imam Hospital. Meanwhile, 85% of strains were detected as ESBL-producers. More than 80% of remaining non-ESBL isolates were recovered from Loghman Hospital. The distribution of the isolates resistant to different number of antibiotics was consistent across all age groups.

### CONCLUSION

Antibiotic resistance in uropathogenic *E. coli* is on the rise, affecting even infants and neonates. The pattern of resistance varies between hospitals and is unrelated to gender or age, but rather specific to each hospital.

**Keywords:** Urinary tract infection, *E. coli*, antibiotic resistance, multidrug resistant, pediatrics

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigation of the frequency of bacteriuria and crystalluria in urine samples of people living in Noorabad, Lorestan

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Urinary tract infections and the presence of stones in the urinary tract are common diseases among people. Many factors caused urinary tract infections and urinary tract stones. To prevent and even treat the disease early, screening in each area can be helpful.

#### MATERIALS AND METHODS

In this study, 370 urine samples from the Noorabad region of Lorestan were analyzed in terms of biochemistry, microbiology and microscopy. Culture media and subtractive tests were used to detect the type of contaminating bacteria.

#### RESULTS AND DISCUSSION

Finally, by analyzing the data, 21.35% of people were involved in urinary infection, which included Gram-negative bacteria, *Escherichia coli* (73.4%), *Klebsiella* (10.5%), *Citrobacter* (5.7%), and Gram-positive bacteria. They included *Staphylococcus* (7.3%) and *Enterobacter* (3.1%). The most common type of bacteria found was *Escherichia coli*. Also, 11.89% of people showed urinary crystal excretion, and the most common type of crystals observed were calcium oxalate (79.54%), amorphous urea (11.42%), amorphous phosphate (6.81%) and uric acid (2.27%). The total number of urine leukocytes and the total number of bacteria in the urine samples were higher in women than in men. The number of leukocytes and the presence of crystals were related to the presence of bacteria and this relationship was significant for all two factors ( $p < 0.05$ ).

#### CONCLUSION

Bacterial infection was more in women than men and the most important reason of infection was *Escherichia coli*. The crystalluria was not different in men and women, and calcium oxalate was the most important crystal in samples. Bacterial infection and the presence of crystals were not related to age.

**Keywords:** Urinary tract infection, Crystalluria, Lorestan

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibacterial effects of the biosurfactant produced by *Sporisorium* sp. aff. *sorghii* SAM20

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Biosurfactants are amphiphilic compounds that are mainly produced by microorganisms. Mannosylerythritol lipids (MELs), which are glycolipid biosurfactants produced by some yeast strains, show not only excellent interfacial properties but also versatile biological activity. The yeast *Sporisorium* sp. aff. *sorghii* SAM20 was previously identified as a producer of unique pattern of MEL mixtures. This study aimed to investigate the antibacterial activity of the biosurfactant mixture against some reference bacteria.

### MATERIALS AND METHODS

The strain was cultured in a medium containing 4% corn oil as carbon source at 28 °C for 7 days. The surface activity of the broth was confirmed by oil displacement assay. The biosurfactants were recovered and partially purified by ethyl acetate and then methanol-hexane extraction. The antibacterial activity of the biosurfactant mixture was determined at different concentrations from 0 to 2000 mg/l by broth microdilution method according to CLSI (Clinical and Laboratory Standard Institute) guidelines and the growth inhibition percentages were calculated.

### RESULTS AND DISCUSSION

The biosurfactant mixture did not completely inhibit the bacterial growth at the applied concentrations. *Bacillus cereus* ATCC 11778 was the most sensitive bacterium to the biosurfactant with 73-98% growth inhibition in the selected concentrations. The growth inhibition range of *Staphylococcus aureus* subsp. *aureus* ATCC 33591 was 38-81%, while the inhibition of *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 10145, and *Salmonella enterica* subsp. *enterica* ATCC 13076 were <50% in different concentrations.

### CONCLUSION

The biosurfactant mixture produced by *Sporisorium* sp. aff. *sorghii* SAM20 showed antimicrobial effects particularly against Gram-positive bacteria. According to the results, the biosurfactants may have potential application as a preservative in food and cosmetic products.

**Keywords:** Antibacterial effect, Biosurfactant, Mannosylerythritol lipids, *Sporisorium*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Optimization of *Spirulina* algae culture medium by response surface method (RSM)

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Spirulina* algae (*Arthrospira platensis*) has gained considerable attention for its nutritional benefits and diverse industrial applications. To harness its full potential, optimizing the culture medium is essential for promoting robust growth and maximizing biomass productivity. This study focuses on culture medium optimization using RSM and the exploration of mixotrophic culture to further enhance *Spirulina*'s productivity.

### MATERIALS AND METHODS

The study employed a systematic approach to optimize the culture medium for *Spirulina* algae using RSM. A Box Behnken Design (BBD) was implemented to investigate the interactive effects of critical factors on *Spirulina* growth and biomass accumulation. Key parameters including NaNO<sub>3</sub>, NaCl and K<sub>2</sub>HPO<sub>4</sub> were investigated in this experiment and the experiments were designed with the help of Minitab Statistical software (V21). Next, biochemical parameters including biomass, protein, etc. were measured.

### RESULTS AND DISCUSSION

The RSM-optimized culture medium enhanced *Spirulina*'s growth and biomass productivity. The interactive effects of the key factors were analyzed, leading to the identification of the optimal nutrient composition for sustained cultivation. The optimized culture medium resulted in higher biomass yield and enriched biochemical content, including proteins and pigments contributing to its value as a nutritious food source. Mixotrophic conditions stimulated enhanced growth rates and metabolic activities; It can also increase the risk of contamination of the cultivation environment.

### CONCLUSION

The comprehensive study demonstrated the significance of employing RSM in optimizing the *Spirulina* culture medium to maximize growth and biomass productivity. The integration of sustainable nutrient sources, such as organic carbon in mixotrophic culture, further enhanced *Spirulina*'s potential as a valuable microorganism. These findings contribute to the advancement of *Spirulina* cultivation techniques, fostering its integration into diverse sectors and promoting a more sustainable future.

**Keywords:** Optimizing culture media, *Spirulina* algae, Random surface methodology (RSM), Mixotrophic culture

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Production of arginase deaminase enzyme for clinical approach

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Instructor, Production of arginase deaminase enzyme for clinical approach

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Arginase deiminase is an enzyme that breaks down arginine. Arginine is an essential amino acid for the growth of cancer cells. This enzyme is used to treat Alzheimer's disease and also to treat some cancers such as hepatocellular carcinoma, melanoma, and mesothelioma. Due to the wide application of this enzyme in industry and medical sciences, we produced this enzyme in a recombinant form.

#### MATERIALS AND METHODS

In this research, the arginase deiminase enzyme gene was cloned into the pet21 vector, then the PCR technique was used to confirm the presence of the desired gene in the bacteria. Then it was transformed by the heat shock method in Escherichia coli strain BL21 and using IPTG with different concentrations and temperatures. The expression of this enzyme was optimized.

#### RESULTS AND DISCUSSION

To confirm the transformation process, first, the growth of bacteria was checked in the culture medium containing kanamycin and the positive transformation samples grew, unlike the negative transformation samples. PCR results also indicate the success of cloning and enzyme transformation. After confirming the presence of the arginase deiminase gene in Escherichia coli strain BL21, the results showed proper protein expression.

#### CONCLUSION

Since the enzyme arginase deiminase was produced in a good way. Therefore, in the next steps, it can be produced on a larger scale to be used for therapeutic purposes.

**Keywords:** Arginase deiminase, Recombinant enzyme, Expression enzyme

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Machine Learning and AI at the Service of Microbiologists

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Machine learning is a branch of Artificial Intelligence that can learn and adapt without following explicit instructions. In the last decade, machine learning allows the analysis of complex and large data sets and has the potential to improve healthcare. A total of 97 ML systems are aiming to assist clinical microbiologists. About 85% of these systems targeted bacterial infections, 11% for parasitic infections, 9% for viral and 3% for fungal infections, respectively.

### MATERIALS AND METHODS

In this review article, the data was collected from international scientific databases, including Google Scholar, Scopus, PubMed, and Elsevier. The key words “Machine Learning”, “Artificial Intelligence” and “microbiology” were searched.

### RESULTS AND DISCUSSION

Machine learning focuses on using data and algorithms to imitate how humans learn, gradually improving its accuracy. The ML systems used very diverse sources of data: 22% used WGS of microorganisms, 20% used microbiota data obtained by shotgun metagenomic sequencing, 20% analyzed microscopic images, 18% used spectroscopy data, 8% used targeted gene sequencing, 6% used volatile organic compounds, 4% used photographs of bacterial colonies, 4% used transcriptome data, 3% used protein structure and 3% used clinical data, respectively.

### CONCLUSION

It is suggested to use ML systems for the diversity of research activities that needs to analyze large data sets. It is a great step to improve healthcare and diagnosis systems.

**Keywords:** Machine learning, Artificial intelligence, Healthcare, diagnosis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Cloning and optimizing the expression of asparaginase enzyme from *Bacillus subtilis*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

L-Asparaginases (L-ASNase, EC 3.5.1.1) are enzymes that catalyze the hydrolysis of L-asparagine to L-aspartic acid and are found in various organisms from microorganisms to mammals. However, they are mainly expressed and produced by microorganisms. Microbial L-asparaginases have received ongoing attention because of their unique role in the treatment of acute lymphoblastic leukemia and because they inhibited acrylamide formation during food processing.

### MATERIALS AND METHODS

The asparaginase enzyme gene was amplified from *Bacillus* bacteria and placed in the pet26 vector by heat shock method and transformed into BL21 bacteria and its expression was induced by IPTG.

### RESULTS AND DISCUSSION

To confirm the methods of bacterial growth in two culture mediums containing the drug kanamycin and without the presence of this drug, it was observed that the transformed bacteria grew in the culture medium containing kanamycin and this method was confirmed by PCR and electrophoresis techniques.

### CONCLUSION

Since the asparaginase enzyme was produced well in this part of the research, it can be used to advance therapeutic goals in other aspects of research.

**Keywords: Cloning, Expression, Enzyme, Asparaginase**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation of serological prevalence of HTLV-1 in Rheumatoid arthritis patients

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### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The precise cause of autoimmune diseases is unknown. However, there are risk factors that may increase the chances of getting it, such as viral infections. Recent studies have suggested a potential link between Human T-Cell Lymphotropic Virus Type 1 (HTLV-1) infection and certain autoimmune disorders. However, the association between HTLV-1 and RA remains poorly understood. Understanding the potential association between HTLV-1 and autoimmune diseases is crucial for developing targeted therapeutic interventions. This study aimed to investigate the serological prevalence of HTLV-1 in one of the autoimmune diseases namely rheumatoid arthritis (RA).

#### MATERIALS AND METHODS

Serum samples from 75 patients with RA and 75 healthy controls were collected for this cross-sectional study. Enzyme-linked immunosorbent assay or ELISA (Dia.Pro, Italy kit), a widely used serological test, was used to detect the presence of HTLV-1 antibodies in the samples.

#### RESULTS AND DISCUSSION

Only 10 out of the 60 samples from the RA patients showed positivity for HTLV-1, while none of the healthy control samples exhibited the presence of HTLV-1 antibodies. Despite the limited number of HTLV-1-positive samples, these findings suggest a possible association between HTLV-1 infection and the development of RA.

#### CONCLUSION

This study presents preliminary evidence suggesting a possible association between HTLV-1 infection and rheumatoid arthritis. These findings emphasize the importance of considering HTLV-1 infection as a possible contributing factor in the complex etiology of autoimmune diseases. Further research is essential to clarify the exact role of HTLV-1 in the pathogenesis of RA, the molecular mechanisms underlying this association and paving the way for more targeted and effective therapeutic approaches for affected patients.

**Keywords:** Human T-Cell Lymphotropic Virus Type 1, HTLV-1, Rheumatoid Arthritis, Autoimmune diseases

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of diazinon pesticide biodegradation efficiency by native bacterial strains isolated from contaminated soils in agricultural areas

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Diazinon is one of the most widely used organophosphorus pesticides in agriculture. The accumulation of this pesticide in agricultural soils and its intrusion into water sources pose a risk to public health and the environment. The aim of this study was to investigate the efficiency of bioremediation of diazinon pesticides by indigenous bacteria isolated from contaminated soils.

### MATERIALS AND METHODS

Soil samples were collected from a tomato greenhouse in Meybod city. The soil samples were cultured in a mineral nutrient medium containing 10% (v/v) diazinon as the sole carbon source. In order to adapt the isolates to higher concentrations, culture media containing 20-70% diazinon were used in the next experiments. The growth rate of the isolates was determined by measuring the optical density of the samples with a spectrophotometer at a wavelength of 620 nm. The synergistic effect of the isolates in the biodegradation of diazinon was also investigated. Superior isolates were identified by biochemical and polymerase chain reaction (PCR) assays. The amount of the pesticide diazinon remaining in the culture medium was measured by gas chromatography (GC) with flame ionization detector (FID).

### RESULTS AND DISCUSSION

Culture of the isolates showed that there were two superior isolates in the soil samples that could grow in a saline medium containing 60% (v/v) diazinon. The results of biochemical tests and partial 16SrRNA gene sequence analysis and blast showed that the two best strains belonged to *Pseudomonas aeruginosa* and *Entrobacter huaxiensis* with 94.5% and 94.7% similarity, respectively. The results of GC analysis showed that *Ps. aeruginosa* and *E. huaxiensis* were able to degrade 87.45% and 75.04% of diazinon, respectively. Simultaneous cultivation of these two species in the culture medium containing diazinon showed no synergistic effect and resulted in the degradation of only 44.34% of diazinon.

### CONCLUSION

The species of *Ps. aeruginosa* and *E. huaxiensis* are suitable candidates for the biodegradation of diazinon in contaminated soils. Biodegradation efficiency decreases in the presence of both bacteria, probably due to the release of secondary metabolites. In order to increase the biodegradation efficiency of diazinon, physical and photocatalytic solutions can be considered along with the use of bacteria.

**Keywords:** Biodegradation, Diazinon, Pesticide, *Pseudomonas aeruginosa*, *Entrobacter huaxiensis*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The use of probiotics as medicinal supplements in the treatment and prevention of some diseases

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The industry of producing super-beneficial foods by adding or concentrating useful compounds and removing ineffective or harmful substances was created and quickly marketed. The production and consumption of these products was developed. Extra-beneficial foods are products that, in addition to providing basic nutrition, increase the health level of people, and according to the consumption of consumers, improvements are made day by day in the production of such foods. In super-beneficial foods, among foods containing probiotic microorganisms, they are especially important. And day by day, there are improvements in the production of such foods. Probiotics are currently being implemented as the best food products, which are being developed and developed by nutritionists. The purpose of this review study is to discuss the past researches about functional foods and the use of probiotics to produce beneficial foods, as well as the use of probiotics for the treatment of digestive and non-digestive diseases.

#### MATERIALS AND METHODS

According to the various studies conducted, the use of probiotics as a tool and candidate for the treatment of gastrointestinal and non-gastrointestinal diseases and can be used in the treatment of a wide range of diseases such as diabetes, cancer and liver problems.

#### RESULTS AND DISCUSSION

New supplements such as polysaccharides of bacterial origin, types of algae and yeasts are a new field in research related to synbiotic supplements due to their economic efficiency and numerous proven benefits for human health. In addition, they can be combined with Obtained by direct extraction from natural sources or produced by chemical processes, hydrolysis of polysaccharides or by enzymatic and chemical synthesis.

#### CONCLUSION

Probiotic food is a food that contains enough probiotics to produce its beneficial effects at the time of consumption, and it must have characteristics such as that the product itself is healthy or at least not harmful, and it should be possible to produce it permanently, it has favorable conditions in terms of the durability of microorganisms and the addition of probiotics does not cause the loss of sensory or special properties. Several researches have been conducted on the effectiveness of using prebiotics and synbiotic products in improving human health, and its positive effects have been proven in the treatment of digestive diseases, diabetes, etc. Also, today, the focus on the treatment and prevention of new and emerging diseases using biological biopolymers has also increased.

**Keywords: Beneficial Foods, Probiotics, Probiotics, Synbiotics**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Inhibitory effect of *jujube* and *barberry* honeys on the biofilm formation of *Candida albicans*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Jujube* honey originated from jujube tree shows anti-inflammatory and antibacterial effects. *Barberry* honey obtained from *Berberis* spp. (*Berberidaceae*) is commonly used to treat diarrhea and heal wounds. The aim of this study was to investigate the effect of pure *jujube* and *barberry* honey on the inhibition of biofilm formation by the resistant strain of *Candida albicans* which over time, scientists implemented diverse strategies to overcome.

### MATERIALS AND METHODS

Two strains of *Candida albicans*, O31 and G109 that were isolated from the patients under endoscopy from Imam Reza Hospital were obtained to be used in this study. The effect of different concentrations (100-70% v/w) of jujube and barberry honey on the yeasts growth and their biofilm formation ability was assessed by the well-diffusion and microtiter-plate methods, respectively.

### RESULTS AND DISCUSSION

In the well-diffusion assay although no clear inhibition zones were observed, at 100% concentration of both honeys, the growth of recruited yeasts were significantly decreased compare to control. After execution of microplate-titer method and consequent statistical analyses, the results revealed that no biofilm was formed in the presence of all examined concentrations of both honeys.

### CONCLUSION

The study exposed antifungal and anti-biofilm activities of jujube and barberry honeys against both strains of *Candida albicans* which is a common cause of infection of the skin, oral cavity and esophagus, gastrointestinal tract, vagina and vascular system of humans. This result suggests that these honeys might find a place in clinical practice, alone or as part of combination antimicrobial therapies, to treat emerging resistant *Candida albicans* species.

**Keywords:** Honey, *Candida albicans*, Biofilm

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Biological characteristics and anti-biofilm activity of a lytic phage against *Enterococcus faecium*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Enterococcus faecium* is emerging as one of the most common causes of nosocomial infections and due to its resistance to antimicrobial agents this organism is one of the major public health concerns. Bacteriophages are being considered as an alternative therapy to treat infections caused by multidrug-resistant bacteria. The purpose of this study was to isolate and characterize an effective antibiofilm bacteriophage against *E. faecium* isolated from hospitalized patients.

### MATERIALS AND METHODS

The bacteriophage was obtained from the sewage of hospital and its lytic activity was determined using the spot test and double-layer plaque assay. Transmission electron microscopy (TEM) was employed to determine the phage characterization. Furthermore, host range, optimal multiplicity of infection (MOI) and stability tests on different temperature and pH were conducted. The effect of the bacteriophage on 1-3-5-day old preformed *E. faecium* biofilm was assessed using TTC assay.

### RESULTS AND DISCUSSION

According to Electron microscopy observations, the isolated phage belonged to the *Myoviridae* family. Spot testing on the collected isolates indicated that bacteriophage was able to lyse 20 out of 36 isolates (55%). Suitable pH spectra for phage survival was 5–11, at which the phage showed 100% activity. The optimal temperature for phage growth was 30–45°C, with the highest growth at 37°C. The optimal MOI for isolated phage was 0,01. The phage at  $5.6 \times 10^{16}$  pfu/ml,  $2.8 \times 10^{16}$  pfu/ml and  $1.4 \times 10^{16}$  pfu/ml disrupted one, three and five day old biofilm of *E. faecium* isolates.

### CONCLUSION

The high broad host range of the isolated phages is promising to control *E. faecium* infections and can be in the future commercially suitable for treatment as lysate preparations. Animal models of phage-bacterial interaction will be our next step that may help in resolving the multidrug resistant crisis of infections due to the *E. faecium* biofilm.

**Keywords:** Bacteriophage; *Enterococcus faecium*; Phage therapy

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# Antibacterial activity of the biosurfactant produced by *Starmerella bombicola* SAM19

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Sophorolipids (SLs) are glycolipid biosurfactants produced by some yeast strains. They have gained a lot of attention in recent decades due to their unique functional and bioactive properties. *Starmerella bombicola* is the best-known SL producer. This study aimed to investigate the antibacterial activity of the biosurfactant produced by *S. bombicola* SAM19, which previously isolated from the flower of *Tragopogon dubius* in Iran, against some reference bacteria.

### MATERIALS AND METHODS

The yeast strain was cultured in a medium containing 10% glucose, 4% corn oil, 1% yeast extract, and 0.1% urea at 28 °C for 7 days. The surface activity of the broth was confirmed by oil displacement assay. The biosurfactant was recovered and partially purified by ethyl acetate and then methanol-hexane extraction. The antibacterial activity of the crude biosurfactant was determined at different concentrations from 0 to 1000 mg/l by broth microdilution method according to CLSI (Clinical and Laboratory Standard Institute) guidelines and the percentages of growth inhibition were calculated.

### RESULTS AND DISCUSSION

The MIC (Minimum inhibitory concentration) of the crude biosurfactant for *Bacillus cereus* ATCC 11778 was less than 31.25 mg/l, but the growth of other bacteria did not completely inhibit at the applied concentrations. The percentages of growth inhibition of *Staphylococcus aureus* subsp. *aureus* ATCC 33591, *Salmonella enterica* subsp. *enterica* ATCC 13076, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 10145 were 6-89%, 55-78%, 25-73% and 6-13%, respectively.

### CONCLUSION

According to the results, the biosurfactant produced by *S. bombicola* SAM19 showed antibacterial effects and it may have potential application as partial or total substitutes for antibacterial agents and preservatives applied in a variety of commercial products.

**Keywords:** Antibacterial effect, Biosurfactant, Sophorolipids, *Starmerella*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation the effect of acetoacetanilide inhibitor on growth and Beta Carotene production in *Blakeslea trispora*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Blakeslea trispora*, accumulates high amount of  $\beta$ -carotene from the biological sources under sexual interaction conditions. *B. trispora* possesses conserved carotenoid biosynthetic pathway (CBP) and Carotenoids are derived from the five-carbon isoprene units, isopentenyl diphosphate (IPP) via the mevalonate pathway (MVA). In the present work, one of the metabolic inhibitors like acetoacetanilide was used in a defined medium, as repeated batch cultures, to understand their influence on carotenoid biosynthesis. Acetoacetanilide is a preferred acetate analog that inhibits acetoacetyl-CoA synthetase (AACS) in the first step of the carotenoid biosynthesis pathway.

### MATERIALS AND METHODS

*B. trispora* PTCC5278 (-), and PTCC5277 (+), The spore suspension was transferred onto CM17 agar plates with different concentrations of acetoacetanilide (1-1.8g/l). After cultivation at 28°C for 96 hr, the colonies were transferred to fresh medium with same concentrations of acetoacetanilide and repeated for 8 times.

### RESULTS AND DISCUSSION

Despite the decrease in the biomass of the strains grown in the environment containing the inhibitor compared to the original strain, the amount of beta-carotene produced by this strain in one gram of dry biomass is 4 times higher than the amount produced in the original strain. The results showed strain with the ability to grow at higher concentrations of this inhibitor can direct more carbon flux to the carotenoid biosynthesis pathway through the intermediary of acetyl-CoA, and thus, it will be effective in the production of  $\beta$ -carotene.

### CONCLUSION

This study is expected to contribute to the development of information on the tolerance of molds that regularly exposed to forms of metabolic inhibitors.

**Keywords:** *Blakeslea trispora*, Betacarotene, inhibitore, Acetoacetanilide, Mevalonate

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the effect of *Lactobacillus brevis* probiotic bacteria lysate on the growth rate of the AGS cell line

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Probiotics demonstrate functional effects as anti-inflammatory, antioxidants, immune system boosters, antibacterial, anti-diabetic, and anti-cancer. One of these probiotics is *Lactobacillus brevis*, which is able to cause the induction of apoptosis through the effect on the mitochondrial signaling pathway. The purpose of this research is to evaluate the effects of *Lactobacillus brevis* lysate on the growth rate of the AGS cell line after twenty-four hours of cell implantation.

### MATERIALS AND METHODS

*Lactobacillus brevis* was obtained from the Center for genetic resources and approached CFU/mL  $3/5-4/9 \times 10^9$  after implantation. Then, the bacteria were separated using a centrifuge to extract their lysate using a new Sonication method. The obtained lysate was lyophilized to determine the density and 2, 1, 0/5, 0/25, and 0/125 mg/mL were the results. The AGS cell line was cultured and transferred to plate 96 which contained the bacteria's lysate to check cell viability with the MTT technique after exposure to lysate for twenty-four hours.

### RESULTS AND DISCUSSION.

The results illustrate that the viability of the cell was in the lowest density (0.125 mg/mL) for 75% and in the highest density (2mg/mL) for 44%. It can be concluded that increasing the density causes a lack of growth in cells.

### CONCLUSION

The lysate of the probiotic *Lactobacillus brevis* reduces the survival of cancer cells of the AGS cell line. Therefore, it may be used to control and reduce the growth of cancer cells.

**Keywords:** *Lactobacillus brevis*, AGS cell line, lysate, MTT assay

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and characterization of luminescent bacteria from the Caspian Sea

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

In marine environments, luminescent organisms play essential ecological roles and have the potential to offer valuable insights into biotechnological applications. Luminescent bacteria are the most abundant and widely distributed of light-emitting microorganisms. *Vibrio* and *Photobacterium* species are the most luminous bacteria. This study aimed to isolate and characterize luminescent bacteria from the unique and ecologically diverse in the Caspian Sea.

#### MATERIALS AND METHODS

Samples were collected from 22 areas across the Caspian Sea in north of Iran, encompassing both nearshore and offshore regions. The samples were enriched in SWB liquid medium and incubated at 28 °C for 24 hours, then transferred onto SWA agar for screening of the luminescent bacteria. Luminous colonies were detected in dark room via emission of visible light. These isolates were examined in terms of morphological and physiological tests. Molecular analyses including polymerase chain reaction (PCR) and 16S rRNA gene sequencing were employed to identify the isolated bacteria.

#### RESULTS AND DISCUSSION

Preliminary findings indicated that 19 luminescent bacteria were isolated from the Caspian Sea. Among the luminous isolates, MAZ5 and MAZ12 were showed a high emission of visible light. Subsequent analysis of these isolates revealed a diverse array of species, with notable variations in bioluminescent characteristics and genetic profiles. This suggests the existence of distinct luminescent bacteria that have adapted to the specific conditions of the Caspian Sea.

#### CONCLUSION

The results of this study demonstrated that a range of luminescent bacteria from the Caspian Sea was isolated. These findings have implications for biotechnological applications, including the development of biosensors and environmental monitoring tools. Overall, this research advances our knowledge of bioluminescence in the Caspian Sea and offers a foundation for future investigations into the ecological significance and potential biotechnological application of these remarkable luminescent bacteria.

**Keywords: Bioluminescence, luminescence bacteria, The Caspian Sea**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Integrating 3D graphene with CuO nanoparticles and investigation of its Gram-Positive and Gram-Negative antibacterial properties

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Recently, graphene-based nanocomposites have raised significant interest in many different areas, one of which being antibacterial agents where CuO nanoparticle-anchored graphene has shown promising potential. Graphene is considered the most interesting material due to its unique properties, including strong mechanical strength, large surface area, and high resistance to degradation. In this study, a 3D grapheme/CuO nanocomposite was synthesized, and its antibacterial effects on both Gram-positive and Gram-negative bacteria were examined.

### MATERIALS AND METHODS

The anti-bacterial and physicochemical properties of 3D grapheme/CuO nanocomposite were investigated. Furthermore, CuO, 3DG, and 3DG /CuO nanocomposite were treated to *Acinetobacter baumannii* ATCC19606 and *Staphylococcus aureus* ATCC25923.

### RESULTS AND DISCUSSION

The proposed structure for graphene-based nanocomposites was confirmed by physicochemical investigations. The anti-bacterial property of 3D grapheme/CuO was demonstrated using the MIC and Time killing assays. Moreover, 3D grapheme/CuO inhibited *A. baumannii* ATCC 19606 and *S. aureus* ATCC25923 in a dosage and time-dependent manner.

### CONCLUSION

Our findings suggested that 3D graphene/CuO nanocomposite could be employed as an anti-bacterial agent.

**Keywords:** 3D Graphene; *Acinetobacter baumannii*; Antibacterial; Nanocomposite; *Staphylococcus aureus*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of Human Papillomavirus by molecular method about clients of Arad Medical diagnostic Laboratory in Kerman city, Iran

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### ABSTRACT

### INTRODUCTION

Human Papillomavirus (HPV) is an oncogenic DNA virus with more than 200 types. It is estimated to be the most common sexually transmitted infection and its prevalence is the highest among young persons within the first few years after sexual debut. The important Low-risk (LR) HPV types are (6, 11, 40, 42, 43, 44, and 54), but the most important High-risk (HR) HPV types are (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59). More than 50% of cervical cancers are related to HR-HPV16. It is also effective in enhancing the incidence of cervical intraepithelial neoplasia (CIN) grades I, II, III. Accordingly, the aim of this study was to evaluate the prevalence of HPV by molecular method in Kerman.

### MATERIALS AND METHODS

This descriptive epidemiological research was conducted during spring 2023 about clients of Arad Medical Diagnostic Laboratory, Kerman, Iran. Pap smears were taken from women with 20 to 60 years old. DNA extraction was performed related to 200 samples by Semi-automatic extraction machine using the relevant kit instructions. Extracted DNA of the HPVs were monitored (Genotyping) by real-time PCR method to detect 34 different types of HPVs by using 4 types of probes.

### RESULTS AND DISCUSSION

The proportion of high-risk (HR)-HPV was 85.71%. The most common HR-HPV types were HR-HPV51 (37%), HR-HPV56 (24%), HR-HPV59 (13%), HR-HPV16 (7.8%), HR-HPV52 (5.2%), and HR-HPV18 (5.2%). The only low-risk (LR)-HPV type was observed as LR-HPV6 (7.8%). Three patients had also colposcopy test which one of them showed dysplasia of CIN1.

### CONCLUSION

A large-scale screening of HR populations is very important and critical. We believe that HPV vaccines, regular education and physical examinations are significant impacts on the prevention of HPV-related diseases. This research includes an updates and highlight on the prevalence of HPV types related to the women of Kerman (South of Iran) during spring of 2023.

**Keywords:** Human papilloma virus (HPV), Real time PCR, Genotype, Cervix cancer.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The inhibitory effect of *Anvillea garcinii* methanolic extract on growth and biofilm formation of *Staphylococcus aureus*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Staphylococcus aureus* causes a wide range of diseases including skin infections, bacteremia, respiratory tract infections, and endocarditis. The pathogenicity of *S. aureus* is dependent on the combined effect of extracellular factors, toxins along with the ability of it to form biofilms. The aim of this study was to investigate the inhibitory effect of *Anvillea garcinii* methanolic extract (AGME), a native plant of Iran, on the growth and biofilm formation of *S. aureus* at the phenotypic and molecular levels.

### MATERIALS AND METHODS

Microdilution and Crystal Violet Staining Assay were used to evaluate the antibacterial and anti-biofilm activity of AGME against *S. aureus* ATCC6538 respectively. To assess the effect of methanolic extract on the expression of genes involved in biofilm formation, *SarA*, *spa*, and *icaA*, overnight culture of *S. aureus* was treated with the plant extract at 4, 16, and 48 hours, followed by Real-time PCR to examine changes in gene expression.

### RESULTS AND DISCUSSION

AGME at concentrations  $\geq 1$  mg/ml was able to inhibit 100% of *S. aureus* growth and had bactericidal activity at a concentration of 3 mg/ml. The highest percentage of inhibition of biofilm formation was observed at a concentration of 0.25 mg/ml methanolic extract. The expression of *spa*, *Sar A*, and *ica A* genes significantly decreased at concentrations of 0.5 mg/ml, 0.25 mg/ml, and 0.25 mg/ml, respectively.

### CONCLUSION

AGME has a significant inhibitory effect on the growth and biofilm formation of *S. aureus*. Further studies are necessary for the clinical application of this extract.

**Keywords:** *Staphylococcus aureus*, Anti biofilm, *Anvillea garcinii*, *spa*, *Sar A*, *ica A*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Molecular detection of toxins A, B and E of *Clostridium botulinum* in botulism suspected specimens

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Botulism is caused by *Clostridium botulinum* toxins (BoNT), which is produced by improper use of canned goods, dairy products, and fish. *C. botulinum* toxin has seven distinct serotypes (A-B-C-D-E-F-G) that have a similar structure but differ antigenically. Serotypes A, B, and E are associated with human clinical cases. Today, in Iran, a definitive diagnosis of botulism is made by identifying the bacterial toxin using the laboratory mice (mouse bioassay). As botulism is a life-threatening condition, a rapid diagnosis is required for successful therapy. The rapid and accurate disease diagnosis techniques and methods play an effective role in saving the lives of those affected. In addition to being fast, accurate, and economical, these diagnostic methods must have high specificity and sensitivity. The purpose of this research is to use the Real-time PCR molecular method to detect *C. botulinum* bacteria in a clinical sample.

#### MATERIALS AND METHODS

In 6 months, 50 suspected clinical samples (feces, patient's stomach secretions, and food samples), sent to the Pasteur Institute of Iran, were collected. After 48 hours of cultivation in Cook-meat medium and under anaerobic conditions, DNA was extracted and SYBR Green Real-time PCR was done for the genes of toxins A, B, and E and the results were analyzed.

#### RESULTS AND DISCUSSION

In this research, out of the 50 samples, 68% were genetically positive. The frequency of toxins B, E, BE, A, and AB of *C. botulinum* was 44%, 32%, 22%, 16%, and 10%, respectively. Mouse bioassay was positive for 6 specimens. The difference between the results of the SYBR Green Real-time test compared to the Mouse bioassay can be due to the high sensitivity of the molecular test to the phenotype as well as the lack of expression of toxin genes in some samples.

#### CONCLUSION

Due to the high sensitivity and accuracy of the SYBR Green Real-time method and the rapid diagnosis of this method, it is recommended to use this method along with the Mouse bioassay method to detect botulism toxin.

**Keywords:** *Clostridium botulinum*, Botulism, Real-time PCR

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# ***Staphylococcal* Cassette Chromosome mec (SCCmec) typing and role of SCCmec in resistance to the tetracycline in *Staphylococcus* isolated from clinical samples in Shiraz**

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The human pathogen *Staphylococcus* isolates are the most common pathogen that causes hospital and community acquired infections. Studying on drug resistance, it is important strategy to prevent these types of infections. The use of molecular typing methods is essential for determining the origin of the strains and also in epidemiological investigations. This study aimed to evaluate the different SCCmec types among staphylococcal clinical isolates, and determination of the relationship between SCCmec typing and molecular resistance to tetracycline.

### MATERIALS AND METHODS

This cross-sectional study was carried out on a total of 93 staphylococcal isolates collected from clinical specimens from Shiraz. Antibiotic susceptibility profiles were identified by agar disc diffusion method according to CLSI guidelines. Bacterial DNA was extracted by using the phenol-chloroform extraction method. The genotypes of SCCmec in the isolates were determined by PCR and the results were analyzed using the chi-square. Strains isolated were tested by PCR to determine the frequency of mec gene, SCCmec types and genes coding for antibiotic resistance.

### RESULTS AND DISCUSSION

Out of 93 samples, 26 samples (27.9%) were infected with coagulase positive *Staphylococcus aureus*, and 67 samples (72.1%) were infected with coagulase negative *Staphylococcus* strains. Assigned SCCmec types by PCR revealed SCCmec type II was predominant type with (60%) samples and followed by SCCmec types I (49%), IVa (42.8%), V (42.8%), IVc (41.3%), IVb (39.7%), IVd (39.7%), and III (38%). Results of antibacterial susceptibility tests for *Staphylococcus* isolates shown that 15 (57.7%) of *Staphylococcus aureus* and 41 (61.2%) of coagulase negative *Staphylococcus* isolates were resistance to tetracycline. Also the significant differences between SCCmec type II with other types was observed.

### CONCLUSION

A mobile genetic element, *staphylococcal* cassette chromosome mec (SCCmec), plays an important role in *staphylococci* pathogenesis. Our findings show that clinical isolates of *Staphylococcus* carrying various types of SCCmec types. The frequency of isolates containing type II in the current study can indicate an emergence of this SCCmec type in the studied medical centers.

**Keywords:** *Staphylococcus*, SCCmec typing, antimicrobial susceptibility, tetracycline, mec gene.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Comprehensive review on gene therapy for colorectal cancer treatment using oncolytic viruses

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Colorectal cancer (CRC) is the third most common cancer globally and a leading cause of death. In 2018, there were 1.8 million cases and 880,792 deaths, with a rising prevalence among those under 50. Conventional therapies like surgery, chemotherapy, and radiation are considered the gold standard. However, additional alternative methods such as Gene-targeted therapy, oncolytic viruses (OVs) offer potentially effective tumor treatment approaches that might shrink tumors and activate anti-tumor host immune responses.

### MATERIALS AND METHODS

Common databases including PubMed, Google Scholar, and Scopus were used for the literature search on oncolytic viral therapy.

### RESULTS AND DISCUSSION

Results from preclinical and clinical investigations focused on the OVs as a possible new therapy option for CRC. These studies have shown that OVs might cause tumor regression, increase survival time, and activate anti-tumor immune responses. OVs have occasionally demonstrated success in individuals who had previously failed other medications, indicating that they might be able to overcome resistance to conventional therapies. However, more study is required to determine the patient categories most likely to benefit from this course of treatment and to optimize the use of OVs in CRC. Currently, poxviruses, reoviruses, herpes simplex viruses (HSV), and adenoviruses are the most frequently employed viruses in experimental cancer research. As a result, OVs offer a potential useful method for undoing the immunosuppression of the tumor microenvironment and making the tumor susceptible to immune checkpoint blockage. Additionally, transgenes may be added to the OV genome via viral genome engineering methods, where the expression of virally encoded genes can immunomodulate tumors.

### CONCLUSION

A potential new method for the treatment of CRC is the use of OVs. In comparison to conventional cancer treatments, OVs have several benefits, including the capacity to specifically target cancer cells while sparing healthy cells and the potential to elicit systemic anti-tumor immune responses. However, there are drawbacks to using OVs, and further investigation is required to maximize their effectiveness in CRC treatment. In general, using OVs may revolutionized cancer treatment and enhance outcomes for CRC patients.

**Keywords:** Oncolytic viruses, Colorectal cancer, Gene therapy, Anti-tumor immune responses

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Distribution Probable influence mobile Waves on growth and antibioticale resistance of gene erm A in Staphylococci isolated from ear

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Staphylococcus qalify to have a resistance to Erythromycin, this resestance commonly associated with resistance to other Macrolides. It is found three genes in and coagulase –positive Staphylococci (16SrDNA&Coa) responsible for this resistance MLSB phenotype (Zmantar et al .,2011).Principal aim this study ,is evaluation probable influence mobile waves on growth and antibioticale resistance of genes ermA in Staphylococcus isolated from ear.

The resistance to antimicrobial agents is an increasingly global problem worldwide, especially among nosocomial pathogens. Staphylococci have become one of the most common causes of nosocomial infections (Livermore2000). Furthermore, PCR based molecular methods are often preferred for determination of antibiotic resistance genes (Woodford 2005). Rapid detection of MLSB by PCR for the ermA gene coding forc Erythromycin resistance. (Bergeron 2003). In this study we are detecting ermA in Staphylococcus isolated from ear speicmens at one of the Shiraz Hospital. The principal aim of this study, is evaluation abundance of ermA at two statistics society on mobil and no mobile.

### MATERIALS AND METHODS

150strains of Staphylococcus isolated from ear samples were subjected to PCR. The strains were maintained on blood agar plates and according to the method of boiling(Genomic DNA Extraction )be performed extraction DNA . We were designed Coa ,16SrDNA and ermA primers and evaluated by blast software (ncbi.com).

### RESULTS AND DISCUSSION

After detection of Coa ,16SrDNA and ermA genes by PCR method ,the products were analyzed on agarose gel(1%) electrophoresis.According to SPSS software and statistics table,finally ermA-producing strains isolates showed high resistance to erythromycin, while genotypic screening about MLSB was showed prevalence of ermA gene (65.33%).

### CONCLUSION

Our results have revealed that ermA genes in on mobile group were more prevalent than the in no mobil group among Staphylococcus isolated of ear. It seems then probably presence of ermA genes have significant relative with Erythromycin and Methycilin. Detection ermA have important uses, on their molecular mechanisms and epidemiology and true nature of the efflux pump and methylase Erythromycin ribosom.

**Keywords:** erm, Distribution, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the effect of cheese flavor producing microorganisms on tofu

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Tofu or soy cheese is one of the most important soy products produced from soy milk which is one of the main protein sources consumed by the East Asians. Soy cheese is obtained from the combination of soy milk proteins which is similar to the cheese from dairy milk. Coagulation is made by either the addition of calcium or magnesium salts or lactic acid bacteria (lactic fermentation). This product could promote human health and increase longevity but due to the lack of special flavor it is not accepted by the Iranian consumers and couldn't have found a good position in the Iranian food market. The aim of this study is to optimize the organoleptic properties of tofu including flavor, taste, and texture of tofu by fermentation. Since the main issue is the different taste of tofu, Lighvan cheese which is one of the most fragrant Iranian traditional cheeses was used to obtain lactic acid bacteria.

### MATERIALS AND METHODS

Microorganisms belonged to 14 different genera were isolated in different general and selective media. Lactic fermentation by these isolates was done at different conditions including time and temperature. At end of the fermentation the sensory properties were evaluated.

### RESULTS AND DISCUSSION

The panel test results indicated a positive impact of 5 isolates in producing metabolites effective in the aromatic and flavor properties of the tofu cheese. Also, the optimization of tofu production was done and optimum conditions were reached.

### CONCLUSION

In conclusion, lactic fermentation could improve the organoleptic properties of tofu cheese and could be used to make tofu more popular in the Iranian market. Also, there is a need to compare the technological effects of these strains on the organoleptic properties of soy cheese and local lighvan cheese in the future.

**Keywords:** Tofu, Soy milk, Lactic acid bacteria, Organoleptic properties, flavor

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibacterial activity of porous alumina nanoparticles against 10 clinical isolates of *Staphylococcus aureus*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Staphylococcus aureus* is one of the most important bacteria causing infections in humans. The prevalence of these infections, along with the increase in antibiotic-resistant strains, has become a concerning problem. The use of nanoparticles to combat bacterial infections can serve as an alternative to antibiotics. The aim of this study was to investigate the therapeutic effect of alumina nanoparticles on *Staphylococcus aureus* isolated from clinical samples, considering the growth of antibiotic resistance and exploring new approaches in nanobiotechnology.

### MATERIALS AND METHODS

In this study, 10 strains of *Staphylococcus aureus* isolated from clinical samples were examined based on catalase, coagulase, and mannitol fermentation tests for identification and diagnostic purposes. The antibacterial activity was evaluated using the well diffusion method on Mueller-Hinton agar culture medium. Different concentrations of nanoparticles were prepared in equal volumes of dimethyl sulfoxide and methanol solvent. After incubation at 37 °C for 24 hours, the sensitivity of bacteria was determined by measuring the diameter of the growth inhibition zone, and the minimum inhibitory concentration was determined.

### RESULTS AND DISCUSSION

All isolates of *Staphylococcus aureus* showed sensitivity to alumina nanoparticles, and the average minimum inhibitory concentration was found to be 20 mg/ml.

### CONCLUSION

The effectiveness of alumina nanoparticles against clinical strains of *Staphylococcus aureus* indicates that this substance can be considered as a suitable antimicrobial agent.

**Keywords:** *Staphylococcus aureus*, alumina nanoparticles, Antibacterial activity

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of O serogroup in uropathogenic *E. coli* isolated from diabetic patients in Shahrekord

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### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Urinary tract infection (UTI) is one of the most common hospital infections resulted from uropathogenic *E. coli* colonization in host mucosal epithelium and damages to host tissue. The ability to constitute biofilm plays an important role in virulence of the bacteria. The present study was conducted with the aim of tracking common uropathogenic *Escherichia coli* serogroups causing urinary tract infection in diabetic patients.

#### MATERIALS AND METHODS

In this research 51 *E. coli* isolations of diabetic patients having UTI symptoms collected and approved using biochemical tests and molecular technique to identify common serogroups of uropathogenic *E. coli* polymerase chain reaction (PCR) done using primer pairs.

#### RESULTS AND DISCUSSION

In this research, the highest frequency was related to serogroup O25, which was detected in 16 isolates (31.37%). Serogroup O15 ranked second after serogroup O25, so that it was reported in 12 isolates (23.52%). After serogroups O15 and O25, serogroup O6 had the highest frequency, so that it was reported in 8 isolates (15.68%).

#### CONCLUSION

The results of this research and comparing it with the results of other researchers show that the common serogroups of urinary tract infections in people with diabetes are similar to other people, identifying these serogroups can help control and prevent urinary tract infections.

**Keywords: Uropathogenic *E. coli*, Diabetic patient, Serogroup**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluation of the expression pattern of TSPOAP1-AS1 lncRNA related to interferon signaling pathway in COVID-19 patient

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Coronaviruses are a serious public health concern and in the last two decades and previously two viruses from this family (MERS-CoV and SARS-CoV) caused severe human diseases. Since 2019, SARS-CoV-2, has caused the COVID-19 pandemic and the disease lead to millions of deaths and substantial morbidity worldwide. LncRNA (Long Non-Coding RNA) are a large group of non-translating RNA molecules with more than 200 nucleotides in size that involve in crucial cellular mechanisms in host cells. Various studies have shown that lncRNAs affect the function of the host immune system and immunological processes and play various roles in activation and function of innate and acquired immune cells and regulate cell behaviors through proliferation, differentiation and apoptosis.

#### MATERIALS AND METHODS

In this case-control study, blood samples were taken from 15 patients with COVID-19 and also 15 healthy controls. After total RNA extraction and cDNA synthesis, we used Real-time PCR to evaluate the expression level of TSPOAP1-AS1. The data were analyzed by 2- $\Delta\Delta$ Ct method and curve analysis was also performed to evaluate diagnostic utility of this molecular marker.

#### RESULTS AND DISCUSSION

TSPOAP1-AS1 is a kind of LncRNA that in the studies, it was previously shown that during influenza A virus infection, the expression level of this lncRNA increases both in the nucleus and in the cytoplasm. Recent findings revealed that the induction of TSPOAP1-AS1 by IAV increases through the activation of NF- $\kappa$ B and during infection, LncRNA TSPOAP1-AS1 induces a decrease in IFN $\beta$  expression by accumulating more in the nucleus. No significant difference in the expression level of TSPOAP1-AS1 in COVID-19 patients was observed in comparison to the control group.

#### CONCLUSION

According to the role of lncRNAs in the body and their functions, it was predicted that the amount of TSPOAP1-AS1 LncRNA would change in the body during viral infection with coronavirus.

**Keywords:** COVID-19, SARS-CoV-2, LncRNA, TSPOAP1-AS1

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating the prevalence of colistin resistance and the presence of *mcr-1*, *mcr-2*, *mcr-3* and *pmra*, *pmrb* genes in gram-negative bacteria isolated from respiratory samples of patients with covid-19.

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Ventilator-related pneumonia is one of the most important causes of death in intensive care units (Lahorepur, 2019). Patients with covid-19 are at increased risk of secondary bacterial infections due to the use of invasive catheters and impaired host immunity. So that the death of cases that encountered bacterial co-infection with covid-19 has been reported recently (Ponset-Megmont 1 et al., 2020; Ferguson 2 et al., 2020). Therefore, in this study, we investigated the prevalence of colistin resistance in gram-negative bacteria isolated from the trachea of patients with Covid-19 using phenotypic and genotypic methods.

#### MATERIALS AND METHODS

96 isolates of Gram-negative bacteria were isolated from the trachea samples of patients with Covid-19 in Al-Zahra Hospital, Isfahan. Microbial sensitivity test for twelve different antibiotics was investigated by standard Kirby Baer method. Determining the minimum inhibitory concentration of colistin was checked by broth microdilution method and the presence of *mcr-1*, *mcr-2*, *mcr-3*, *pmra* and *pmrb* genes was identified by PCR method and if it was positive, sequencing was done.

#### RESULTS AND DISCUSSION

In this study, the most isolates (37.5%) were isolated from the ICU department and the least isolates (13.9%) from the surgical department. The isolates included 73% *Acinetobacter baumannii*, 16.2% *Klebsiella pneumoniae*, 6.7% *Pseudomonas aeruginosa* and 4.1% *Escherichia coli*. The highest resistance to ampicillin (94.6%) and the lowest resistance to gentamicin and ceftazidime with 74.3% frequency were observed. Based on the MIC results, the average MIC concentration of the antibiotic Cleistin was 13.7 µg/ml. The lowest level of resistance was observed at concentrations of 32, 64, and 128 µg/ml (1.4% of isolates) and the highest level of resistance to the antibiotic colistin was observed at concentration of 1 µg/ml (23% of isolates). 18.9% (14.74) isolates had *pmrAB* resistance gene and no colistin resistance genes (*mcr-1*, *mcr-2* and *mcr-3*) were found in any of the isolates.

#### CONCLUSION

In this study, the level of resistance to colistin in gram-negative bacteria isolated from patients with covid-19 was relatively high, and because colistin is the last treatment option for gram-negative bacteria, resistance to colistin is a serious problem all over the world. Therefore, it is very important to obtain information about colistin resistance and continuous monitoring to determine the frequency of different genes, because these data can be used to create appropriate guidelines regarding the special use of this antibiotic to prevent resistance to colistin.

**Keywords:** Covid-19, Gram-negative bacteria, Resistance to colistin, *mcr-1*, *mcr-2*, *mcr-3*, *pmra* and *pmrb*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigation of *mphA* gene in *Shigella* isolates from stools

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Shigellosis is endemic worldwide and often causes bacterial dysentery. Antibiotic treatment is a critical approach to prevent the spread and mortality of bacterial infection. azithromycin is used as an alternative treatment for *Shigella* species that are resistant to multiple drugs simultaneously. *mphA* is a gene that is resistant to azithromycin. Through plasmid transfer, *Shigella* species acquire the mechanism of macrolide resistance. The present study evaluates the distribution of the azithromycin resistance gene in clinical isolates of *Shigella*.

#### MATERIALS AND METHODS

Faecal samples (n = 50) were collected from a general hospital in Tehran, Iran. After isolating *Shigella* isolates and determining their identity, DNA extraction was performed by boiling method, molecular study based on PCR was performed to confirm the presence of resistance genes in *Shigella* isolates.

#### RESULTS AND DISCUSSION

32(64%) isolates were *shigella sonnei* and 13(26%) isolates were *shigella flexneri* and 3(6%) isolates were *shigella dysenteriae* and 2(4%) isolates were *shigella boydii*. According to the PCR results, out of fifty isolates, fourteen isolates (28%) contained the *mphA* gene.

#### CONCLUSION

Investigation of the characteristics of *Shigella* as a cause of diarrhoea and awareness of the pattern of antibiotic resistance helps to reduce the prevalence of infection and the cost of treatment. As azithromycin is recommended for the treatment of multi-resistant *Shigella* infections, the susceptibility of these isolates to azithromycin should be tested regularly. Treatment of *Shigella* infections with oral antibiotics remains difficult due to the persistence of multidrug resistance. Determining the susceptibility profiles of azithromycin is essential.

**Keywords:** *Shigella*, antibiotic resistance, azithromycin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antimicrobial effect of essential oil, nano-emulsion of essential oil and green synthesis of copper (Cu) nanoparticles produced from aqueous extract of *Salvia mirzayanii*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nowadays, due to the resistance of bacteria to conventional antibiotics and antimicrobial agents, many researches are being conducted to find new types of effective antimicrobial agents. Copper has been increasingly used in the form of nanoparticles with high antimicrobial activity and cheap price against all Gram-negative and Gram-positive bacteria. *Salvia mirzayanii* is one of the medicinal species of mint family and its antimicrobial effects have been proven. In this research, the effect of essential oil, nanoemulsion of essential oil and green synthesis of copper nanoparticles produced from the aqueous extract of *Salvia mirzayanii* along with the standard antibiotic gentamicin on *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) was performed.

### MATERIALS AND METHODS

The essential oil of *Salvia mirzayanii* was prepared in the laboratory. Microdilution method was used to check MIC and MBC of essential oil against the three mentioned bacteria. For the synthesis of essential oil nanoemulsion and copper nanoparticles, the aqueous extract of *Salvia mirzayanii* was used in laboratory. The data was analyzed by SPSS version 26 software, descriptive statistics tests, average standard deviation and analytical statistical tests of analysis of variance.

### RESULTS AND DISCUSSION

The results of essential oil analysis showed that the main compounds were phenolic monoterpenes. The essential oil prepared from *Salvia mirzayanii* did not have any inhibitory effect against *P. aeruginosa*, but the average MIC, MBC and ZOI of *Salvia mirzayanii* essential oil against *E. coli* were in the range of 500, 500, 11.4-9.4 microliters/ml, respectively. Also, the MIC, MBC and ZOI of *Salvia mirzayanii* essential oil against *S. aureus* were 250, 250-500 and 13.5-17.5 microliters/ml, respectively. The results showed that in all bacteria, the lowest inhibitory effect was related to the essential oil and the highest inhibitory effect was related to the antibiotic gentamicin. The analysis of produced copper nanoparticles confirmed the green synthesis of nanoparticles in the range of nanoparticles.

### CONCLUSION

The findings of this research showed that the aqueous extract of *Salvia mirzayanii* has a high potential in the production of copper nanoparticles. Also, due to the antibacterial properties of essential oil, nanoemulsion of essential oil and copper nanoparticles biosynthesized from the extract of *Salvia mirzayanii*, it can be used as a supplement to antibacterial drugs.

**Keywords:** copper nanoparticles, *Salvia mirzayanii*, green synthesis, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular Epidemiology of Norovirus in Iran: A Common Cause of Acute Gastroenteritis in Children and Adults

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Acute gastroenteritis (AGE) is a global public health issue and the second leading cause of morbidity. Advancements in sanitation, water purification, and food safety have replaced bacteria as the primary cause of AGE. Noroviruses (NoVs) are one of the most frequent causes of viral gastroenteritis, belong to the Caliciviridae, subdivided into 10 genogroups (GI-GX) that genogroups GI, GII, and GIV infect humans. Molecular epidemiology is crucial for understanding NoV infection spread in Iran. This abstract aims to identify the most frequent genogroups and genotypes of NoV circulating in Iran.

### MATERIALS AND METHODS

Common databases including PubMed, Google Scholar, and Scopus were used for the literature search on NoV molecular detection in Iran.

### RESULTS AND DISCUSSION

According to studies from 2008 to 2022, NoV prevalence in Iranian AGE ranged from 0.6% to 31.17%. NoV genogroup GII is more prevalent, causing severe infections in children which NoV GII and GI identified in 65% to 100% and 13.30% to 35%, respectively. However, none of the studies detected any infection case of genogroup GIV. GII.4, GII.8 and GII.3 are the most common genotypes found in 77.8%, 32% and 13.9% of AGE cases in Iran, respectively, although fewer percentages for GII.2, GII.6, GII.7, GII.17 and GII.12 were identified. A recent study in 2022 demonstrated the newly emerging GII.8, GII.17 and genotype that presents a new pattern of NoV circulation in children less than 5 years of age with AGE in Iran.

### CONCLUSION

This study describes prevalent NoV genotypes in Iran that were similar to other studies worldwide. Despite the decrease in mortality with AGE, the prevalence of enteric viruses has not decreased in recent years. Identification of viruses is not routinely performed in Iran's hospitals and clinical diagnostic laboratories. Understanding the molecular epidemiology of norovirus in Iran can aid in outbreak investigation and prevent further spread of the disease. Due to low infection doses, high viral excretion rates by infected individuals, the emergence of novel NoV genotypes, and the presence of the virus in contaminated food and aquatic environments, further studies are critical to characterizing the NoV circulating in the general population and high-risk environments.

**Keywords:** Molecular epidemiology, Norovirus, Acute gastroenteritis, Iran.

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# Investigating the antifungal activity of silver nanoparticles biosynthesized by *Aloysia citrodora* plant extract in controlling *Pythium aphanidermatum* in laboratory conditions and investigating the physico-chemical characteristics of biosynthesized nanoparticles

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Silver nanoparticles are of considerable importance due to their antimicrobial properties, and due to their simplicity of synthesis, low toxicity, and high stability, they are suitable for various applications in many fields, including medicine, pharmaceuticals, agriculture, etc. Researchers have shown the unique role of plants in the biosynthesis of these nanoparticles. The present study investigated the ability of *Aloysia citrodora* plant in the biosynthesis of silver nanoparticles and the inhibitory effect of these nanoparticles on the oomycete *Pythium aphanidermatum* under laboratory conditions and investigated the physico-chemical characteristics of these nanoparticles.

### MATERIALS AND METHODS

The ability of *Aloysia citrodora* plant extract to produce nanoparticles was investigated based on visual observations, and the effect of biosynthesized nanoparticles on the inhibition of *P. aphanidermatum* oomycete at different concentrations of silver nanoparticles, *in vitro*. In order to evaluate the physico-chemical characteristics of nanoparticles, TEM, UV-Visible spectroscopy and DLS analyzes were performed.

### RESULTS AND DISCUSSION

The color change of silver nitrate solution from pale green to brown indicated the biosynthesis of silver nanoparticles. The inhibition of biosynthesized silver nanoparticles in concentrations of 0.01, 0.005, 0.002, 0.001 and 0.000625 M was investigated in laboratory conditions. The concentration of 0.01 had complete inhibition and *Pythium* species; while it had a lower growth rate in concentrations of 0.005, 0.002 and 0.001 M compared to control. The growth of *Pythium* at the concentration of 0.000625 was the same as the control. The absorption peak of silver nanoparticles was at 450 nm and the average particle size was 75 nm.

### CONCLUSION

In general, the synthesis of nanoparticles by plants is environmentally safe procedure. In terms of the future, it is important to study the quality of their use in non-chemical approaches to manage fungal infections. In general, the use of silver nanoparticles in the fields of controlling human, animal and plant diseases, the protocols require the use of the appropriate standards to maintain sustainable environment, and therefore it is recommended to investigate silver nanoparticles in the fungal disease control before the aforementioned supplementary studies.

**Keywords:** Silver nanoparticles, *Pythium aphanidermatum*, fungal diseases, biological control

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Outer membrane proteins-focused pseudomonas aeruginosa vaccine designed using reverse vaccinology

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Antibiotic resistance is recognized as a major threat to global health that can result in increased morbidity and mortality. On February 27, 2017, the first list of antibiotic-resistant priority pathogens was published by the World Health Organization (WHO). These pathogens, including *Pseudomonas aeruginosa*, pose the greatest threat to human health. This research was conducted in order to introduce viable candidate vaccine proteins against *Pseudomonas aeruginosa* outer membrane proteins (OMPs) using reverse vaccinology approaches.

#### MATERIALS AND METHODS

Fifty-eight clinical isolates and 9982 outer membrane protein sequences were retrieved for vaxign2 calculation of sequence-derived features. First, 30 proteins with the highest adhesion probability were selected, and in the next step, 10 candidates common among the 58 strains with the highest scores are introduced as candidates for further studies. After determining the physicochemical characteristics of these vaccine candidates, the presence of protected domains was predicted in 2 out of 10 proteins.

#### RESULTS AND DISCUSSION

Based on the results obtained from the bioinformatics analysis of the antigenic properties of these proteins, we identified 10 outer membrane proteins that have the highest antigenic scores, are common among all 58 clinical isolates, have no human protein homologs, and have less than 1 transmembrane helix. These candidate proteins have optimal physicochemical properties. And the presence of conserved domains was predicted only in the outer membrane porin F and enterobactin iron receptor.

#### CONCLUSION

We suggested 10 candidate proteins that showed suitable characteristics including outer membrane protein F (OprF) and ferric enterobactin receptor.

**Keywords:** *Pseudomonas aeruginosa*, reverse vaccinology, vaxign2, antibiotic resistance; vaccine candidate, outer membrane proteins, bioinformatics

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and identification of lytic bacteriophage against *Enterococcus faecalis* isolated from root canals of decayed teeth

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Enterococcus faecalis*, one of the most important causes of infection in the root canal of the tooth with a prevalence between 24-77%. Its removal is difficult because it is capable of formation of biofilm and resistant to antibiotics. Sometimes they remain even after treatment with disinfectants and mechanical removal. Therefore, it is necessary to use alternative methods. Bacteriophages can be a powerful, healthy and useful tool. Bacteriophages as a novel and powerful tool can be useful and valuable for treatment-resistant infections. The aim of this study was isolation and identification of a lytic bacteriophage against *Enterococcus faecalis* strains from the decayed tooth canal and determined by biological characteristics.

#### MATERIALS AND METHODS

In this study, a sample was taken from the root canal to isolated *Enterococcus faecalis* and it was cultured in Mitis salivarius agar medium. Suspected *Enterococcus faecalis* isolates were confirmed by biochemical tests and PCR and were deposited in Genebank. A lytic bacteriophage against *Enterococcus faecalis* was isolated from wastewater in East of Isfahan, Iran. The effects of temperature, pH values, NaCl concentrations, adsorption rate, burst size, multiplicity of infection (MOI), efficiency of plating (EOP) and host range were investigated on the bacteriophage. Data were analyzed by GraphPad Prism version 8.0 software (GraphPad Software Inc., USA) using one-way analysis of variance (ANOVA).

#### RESULTS AND DISCUSSION

The bacteriophage was effective against a wide range of Gram-negative and Gram-positive bacteria and showed a high efficiency of plating ( $EOP \geq 0.5$ ) in about 81% of the *Enterococcus faecalis* isolates. The bacteriophage was stabled in an almost wide range of temperature (-20–50°C), pH values (6-11), and NaCl concentrations (1-10%). Also, the bacteriophage was a short latent period (10 min) with a large burst size (125 p.f.u. cell<sup>-1</sup>) and appropriate lytic activity especially at high MOI.

#### CONCLUSION

In this study, we isolated a novel *Enterococcus faecalis* phage with broad lytic activity, which based on biological properties investigated, this bacteriophage can be used as a suitable choice for genomic studies in preparation for commercialization in the direction of phage therapy.

**Keywords:** *Enterococcus faecalis*, lytic bacteriophage, Root canals, Phage therapy

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of factors causing urinary tract infection in patients referred to Imam Khomeini hospital in 2022 Shirvan

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Urinary tract infection (UTI) is one of the most common causes of bacterial infection in the community and hospital. Urinary tract infection is more common in women than in men (1,2). The economic and public health costs resulting from urinary tract infection and the increasing antibiotic resistance are significant and have a large impact on the quality of life of infected patients (3).

### MATERIALS AND METHODS

In this descriptive-cross-sectional study, during a one-year period from April to March 2022, urine samples of outpatients and inpatients referred to Imam Khomeini hospital in Shirvan were collected using the Clean Catch Midstream method. First, the samples were cultured on McConkey agar and blood agar media, and incubated at 35-37°C for 24-48 hours. A colonization rate equal to or greater than 10<sup>5</sup> colonies per ml was considered as a positive sample. Then the morphology of the colonies is examined and standard biochemical tests are carried out including: Urea, SIM, MR/VP, TSI and Simon Citrate, phenylalanine deaminase and warm dyeing were used.

### RESULTS AND DISCUSSION

In this study, 2089 urine samples from outpatients and inpatients referred to the laboratory of Imam Khomeini Shirvan Hospital, 122 urine cultures (5.84%) were positive 92 samples (75.4%) are women and 30 samples (24.6%) are men. The most common bacterial isolate is *Escherichia coli* with 77 (63.11%), *Klebsiella species* with 18 (14.75%), *Staphylococcus aureus* 4 (3.28%), *Streptococcus group B* 4 (3.28%), *Staphylococcus epidermidis* 3 (2.46%), *Enterococcus* 3 (2.46%), *Streptococcus group D* 3 (2.46%), *Enterobacter* 3 (2.46%), *Staph saprophyticus* 2 (1.64%), *Citrobacter* 2 (1.64%), *Proteus mirabilis* 1 (0.82%), *Streptococcus* 1 (0.82%) and *Pseudomonas aeruginosa* 1 (0.82%) were isolated.

### CONCLUSION

According to the obtained results, *Escherichia coli* bacteria is the most common cause of UTI, which is more than other isolates and is the most dominant bacteria in causing UTI. And the number of Gram-negative bacteria, especially Enterobacteriaceae family, is more than Gram-positive bacteria in causing urinary tract infection.

**Keywords:** Urinary tract infection, shirvan, Bacterial isolates

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# CRISPR/Cas13: Revolutionizing Bacterial Detection and Diagnostics

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The emergence of CRISPR/Cas13 technology has revolutionized the field of molecular diagnostics, providing a powerful tool for the detection of bacterial pathogens. With its precision and high sensitivity, CRISPR/Cas13 offers new possibilities in the fight against infectious diseases. In this article, we will explore the applications, advantages, challenges, future directions, and impact of CRISPR/Cas13 in bacterial detection.

#### Applications and Advantages:

CRISPR/Cas13 offers diverse applications in bacterial detection. By targeting specific bacterial RNA sequences, Cas13 protein can be guided to cleave the targeted RNA molecules in a highly specific manner. This enables researchers to create novel diagnostic assays for detecting bacterial infections. The advantages of CRISPR/Cas13 for bacterial detection include its rapidity, scalability, and high sensitivity. It allows for the detection of multiple bacterial species in a single assay, reducing the turnaround time and cost.

#### Challenges:

While CRISPR/Cas13 presents immense potential, it also faces certain challenges. One major challenge is the need for comprehensive databases of bacterial RNA sequences. To accurately identify bacterial pathogens, extensive databases are required to guide Cas13 to specifically recognize and cleave the target sequences. Additionally, the delivery of Cas13 and guide RNA into the target cells poses a technical challenge that needs to be addressed for efficient and safe bacterial detection.

#### Future Directions:

The future of CRISPR/Cas13 in bacterial detection looks promising. Scientists are continuously improving the technology's efficiency and expanding its applications. Efforts are being made to enhance the sensitivity and specificity of CRISPR/Cas13-based assays, opening doors to more accurate and reliable bacterial detection. Additionally, the integration of CRISPR/Cas13 with other diagnostic platforms, such as microfluidics and point-of-care devices, holds the potential for rapid and accessible bacterial detection in various settings.

#### Impact:

The impact of CRISPR/Cas13 in bacterial detection extends beyond the laboratory. Its potential to revolutionize diagnostics and offer rapid, affordable, and accurate detection has implications for public health, clinical settings, and monitoring of infectious diseases. Early and precise identification of bacterial pathogens can lead to timely treatment, reduce the spread of antibiotic resistance, and ultimately save lives. It also has the potential to revolutionize food safety, agriculture, and environmental monitoring.

## CONCLUSION

CRISPR/Cas13 technology has emerged as a game-changer in bacterial detection. Its ability to specifically target and cleave bacterial RNA sequences with high precision and sensitivity offers new avenues for rapid and accurate diagnostics. While challenges exist, ongoing research and innovation hold promise for overcoming these hurdles. The future integration of CRISPR/Cas13 with other diagnostic technologies could lead to accessible and effective bacterial detection methods with significant impacts on healthcare and public health. With ongoing advancements, CRISPR/Cas13 has the potential to transform the way we diagnose and manage bacterial infections, providing a powerful tool in our fight against infectious diseases.

**Keywords:** CRISPR/Cas13 technology, Molecular diagnostics, Bacterial pathogens, diagnostic assays

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Frequency of Class 1 and 2 Integrons in *Escherichia Coli* Strains Isolated from Urinary Tract Infections

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Integrons are movable genetic components that help bacteria transmit and distribute antibiotic resistance genes. The aim of the present study was to determine the frequency of class 1 and 2 integrons in *E. coli* strains isolated from urinary tract infections (UTIs) in northern Iran.

### MATERIALS AND METHODS

One hundred fifty-three clinical isolates of uropathogenic *E. coli* (UPEC) were collected from UTI patients. Cultures and biochemical assays were used to identify these strains. Polymerase chain reaction (PCR) was used to identify integrons using oligonucleotide primers specific for class 1 and 2 integrons.

### RESULTS AND DISCUSSION

Analysis of PCR results for the presence of class 1 and 2 integrons showed that 49.67% of the isolates had class 1 integron, while class 2 integron was observed in 27.45% of the strains. It was also found that 14.37% strains were found to carry both class 1 and class 2 integrons.

### CONCLUSION

*Escherichia coli* isolated from urinary tract infection patients in northern Iran included class 1 and 2 integrons, which can be a public health concern since they can contribute to the transmission and maintenance of antibiotic resistance among the bacterial population in the region. It must be continually checked.

**Keywords:** Class 1 and 2 Integrons, *Escherichia coli* strains, Urinary tract infections, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Determination of antibiotic resistance pattern about clinical isolates of *Pseudomonas aeruginosa* obtained from medical diagnostic laboratories of Kerman city in south east of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa* is the main causes of nosocomial infections with a mortality rate up to 40-50%. Its ability to develop resistance to antibiotics is a global challenge in the treatment of related infections. Its capacity for drug resistance has been reported in multiple locations across Iran. Accordingly, the aim of this study was to isolate, identify and evaluate the pattern of antimicrobial resistance among clinical isolates of *P.aeruginosa* obtained from medical laboratories.

### MATERIALS AND METHODS

Clinical samples from different medical laboratories of Kerman city were checked in spring 2023. Standard microbiological procedures such as oxidase, motility, growth at 42°C were used for isolation and identification of *P.aeruginosa* strains. Kirby-Bauer disk diffusion method was also used for drug susceptibility testing and the interpretation of results was done based on the CLSI guideline. Lack of susceptibility to at least each pathogenic agent in three or more chemical classes of antibiotics was recognized as multidrug-resistant (MDR) *P.aeruginosa*.

### RESULTS AND DISCUSSION

A total of 30 isolates of *P.aeruginosa* were purified and identified according their growth on Nutrient agar as well as the blue, green or brown pigment production, motile oxidase-positive bacteria with unique ability to grow at 42°C. Among 30 strains, maximum resistance was observed related to meropenem (73.3%). Other results revealed that resistance to imipenem, ciprofloxacin, tobramycin, piperacillin-tazobactam, cefepime and ceftazidime was evaluated as 63.3%, 53.3%, 53.3%, 43.3%, 43.3% and 40%, respectively. It is important to note that all strains were sensitive to colistin. Multidrug resistance (MDR) was also observed as 40%.

### CONCLUSION

Drug resistance about above mentioned isolates were observed from low to moderate rates. Meropenem resistance was high irrespective of the site of infection similar to many other researches. This pattern of resistance indicates probable overuse of broad-spectrum antibiotics. Overuse in each institution should be monitored with order for regulating the use of broad-spectrum antibiotics.

**Keywords:** Antibiotic resistance pattern, *Pseudomonas aeruginosa*, Clinical isolates, MDR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antiviral Effect of phenolic compounds extracted from some Iranian honeys against Herpes Simplex Virus Type 1

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Despite the increasing progress in the treatment of viral diseases, problems such as viral resistance, high costs of antiviral drugs, limitations of effectiveness and side effects caused by antiviral drugs have necessitated the use of natural compounds. Honey is used as a natural product in the treatment of ulcers, gastrointestinal disorders, lung disorders, diabetes, heart ailments, bacterial, fungal and viral infections. The antiviral activity of honey is probably related to the phenolic compounds present in it. The main aim of this study was to investigate the antiviral effect of phenolic compounds extracted from honey against HSV-1 virus.

### MATERIALS AND METHODS

In this study, the physicochemical properties, antioxidant activity and phenolic content of 13 Iranian honey samples were investigated. Then three honey samples, based on the best physicochemical properties and the highest phenolic content, including thyme honey, ziziphus honey, and alhagi honey were selected to assay the antiviral activity. In this research, Vero cell culture was used for antiviral activity and cell survival was determined by MTT method.

### RESULTS AND DISCUSSION

The results showed that the phenolic compounds extracted from all three honey samples were effective against the HSV-1 virus. Among the tested honeys, thyme honey and then ziziphus honey showed the highest anti-viral effect, and alhagi honey inhibited the growth of the virus to a lesser extent.

### CONCLUSION

Based on the findings of this research, it was found that the phenolic compounds in honey have antiviral properties against the HSV-1 virus. Further research on other types of viruses and identification of effective phenolic compounds is suggested.

**Keywords:** Iranian honey, HSV-1, Antiviral activity, Phenolic compounds

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# An In silico analysis to study the molecular host-microbe in *Brucella melitensis* infection: inhibition of immune responses by inactivation of GSK3 $\beta$

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Brucellosis is the most common zoonotic disease worldwide. The Gram-negative coccobacillus, *Brucella melitensis*, is identified often as the leading cause of this disease. Treating and preventing brucellosis is challenging because microorganism can induce host cells to provide a favorable environment for proliferation and survival. It destroys the host's immune system, leading to persistent chronic infection. Host-microbe protein interactions play an important role in various diseases. This interaction can alter the host signaling, metabolic, and cellular processes. Recently, the computational bioinformatics analyses have dramatically advanced our understanding of the host-microbe protein interactions. In this study, we used several bioinformatics tools to evaluate the host-bacteria protein interactions, helping us for better understanding the molecular mechanism of *B. melitensis* pathogenesis.

### MATERIALS AND METHODS

The ImitateDB database was used to identify bacterial motifs that interact with human proteins. The *B. melitensis* biotype 1 (strain 16M / ATCC 23456) was selected and proteins interacting with human cells were recognized. Then, host interactor protein IDs were extracted. These codes were converted into gene symbols through BioDBnet database, and then their function was determined using String and EnrichR database.

### RESULTS AND DISCUSSION

The ImitateDB database showed that *B. melitensis* protein, Winged helix-turn-helix domain-containing protein, with an unknown function, mainly interacts with MYD88 (Myeloid differentiation primary response 88) protein. In contrast, this bacterial protein and TcpB (probable 2' cyclic ADP-D-ribose synthase TcpB) bind to the human TIRAP (TIR domain containing adaptor protein) protein. As presented in Figure 1, MYD88 and TIRAP proteins are also related to 28 human proteins through different motifs. Functional analysis by EnrichR revealed that all these human proteins are significantly involved in Toll-like receptor recognition, NF- $\kappa$ B (Nuclear factor kappa-light-chain-enhancer of activated B cells) signaling, and inactivation of GSK3 $\beta$  (Glycogen synthase kinase-3 beta) by Akt (Protein kinase B), causing accumulation of  $\beta$ -catenin in alveolar macrophages (Figure 2). Since the activated GSK3 $\beta$  promotes the activation of NF- $\kappa$ B, leading to a proinflammatory response, it seems *Brucella* inhibits immune signal transduction by inhibiting the GSK3 $\beta$  (2).

### CONCLUSION

Bioinformatics approaches can be used for understanding of the molecular mechanisms of *Brucella* pathogenesis and host protective immunity against *Brucella* infections, and to facilitate vaccine design and development.

**Keywords:** *Brucella melitensis*, Bioinformatics analysis, protein-protein interaction, molecular mechanism

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of lead metal biosorption by *Rhodococcus erythropolis* PTCC1767

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Removal of heavy metals from the environment due to industrial activities is of grave concern due to the toxicity of these metals in humans and other forms of life. Among the toxic heavy metals, lead is in the limelight due to its major environmental impact. Microbial biomass has been identified and documented as an effective factor in metal removal. This work is to determine the biological uptake of lead by *Rhodococcus erythropolis*.

### MATERIALS AND METHODS

A strain of *Rhodococcus erythropolis* was prepared by the Microbial Bank of Iran Scientific-Industrial Research Organization number 1767. The Minimum inhibitory concentration (MIC) for lead metal is measured. Made of stock lead metal. Cells are collected at the end of the exponential phase and then centrifuged. The culture medium discharged and the cell mass was washed three times with saline solution. The biomass was autoclaved at 121 °C for 20 min and dried in an oven at 105 °C for 12 h. Specific concentrations of lead metal were prepared. 0.01 g of dead bacteria sediment was added to the culture medium and metal and placed in an incubator shaker. After 24, 48, and 72 hours, 10 cc of the medium was centrifuged using a refrigerated centrifuge. A supernatant was used to read spectrophotometric and atomic absorption spectrometers.

### RESULTS AND DISCUSSION

The MIC value for lead metal is 250 µg/ml. For the lead element, it happened in 24 hours and at a concentration of 200 ppm with 99.7% by the bacterium *Rhodococcus erythropolis*. The presence of organic compounds, such as carboxylic acids, amides, and ketones, is available for capturing lead ions.

### CONCLUSION

In this study, *Rhodococcus erythropolis* showed the ability of bioremediation of lead metal, considering that ecosystem pollution caused by heavy metals poses many risks to human health and other living organisms, as a result, using bioremediation to remove metals can solve the limitations and problems related to physical and chemical methods.

**Keywords: Biosorption, Lead metal, *Rhodococcus erythropolis* PTCC1767**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Extraction of antioxidant bioactive compounds from Iranian native *Chlorella* sp. microalgae

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

In recent years, the demand for natural antioxidants as an alternative to synthetic antioxidants has increased. Therefore, the microalgae have become a source of molecules for a healthy life. Their valuable compounds like peptides, carbohydrates, lipids, carotenoids, phenolic compounds and vitamins makes them a promising new source of antioxidant molecules. The antioxidant capacity of each microalgae can depend on kind of species and solvent for extraction of various antioxidant compounds.

### MATERIALS AND METHODS

This study aimed to investigate the antioxidant capacity of Iranian native two species *Chlorella* sp. (a) and *Chlorella* sp. (b) microalgae. In order to, the microalgae were extracted using methanol, ethyl acetate and hexane solvent by a three-step sequential extraction procedure.

### RESULTS AND DISCUSSION

The maximum scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (27% and 22% inhibition at 1 mg/mL) were observed for ethyl acetate and methanol extract in (a) and (b) treatments, respectively. In addition, the methanol extract showed the maximum antioxidant capacity in a ferric-reducing antioxidant power (FRAP) activity with a value 964.8  $\mu\text{M Fe}^{2+}$  in (b) treatment and 705.8 in (a) treatment at the concentration of 1 mg/mL. After methanol extract, the maximum antioxidant capacity were observed in the ethyl acetate and then hexane extract, respectively. These results indicated depend on kind of species, the methanol solvent could extract the maximum compounds with the highest FRAP activity.

### CONCLUSION

Therefore, more studies can help to find the best microalgae species and extraction solvent for accessing the highest antioxidant capacity for using in the pharmaceutical, cosmetic, and food industries.

**Keywords:** *Chlorella* sp, 2,2-diphenyl-1-picrylhydrazyl radical, ferric-reducing antioxidant power activity, methanol extraction.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibacterial Effects of Bacteriocin secreted by *Lactobacillus reuteri* against pathogenic *Helicobacter pylori*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Helicobacter pylori* causes gastritis, ulcers and gastric cancer. *H. pylori* has infected more than 50% of the population worldwide. Due to the side effects of antibiotics and strains resistance, the disadvantages of this type of treatment will be greater than its benefits. Reducing *H. pylori* in the stomach by selective interaction of bacterial cells as an effective method to compete with stomach pathogens is optimal and it may be effective as a blockage in the treatment of *H. pylori*

### MATERIALS AND METHODS

In this study, various samples of dairy products in different parts of the country were used to isolate the *L. reuteri* strain. Microscopic observation, biochemical and molecular tests and sequencing were performed to confirm the strain. Molecular and biochemical tests were performed to *H. pylori* identification. Anti-*Helicobacter pylori* activity of *Lactobacillus* strains by three cell-free supernatant (CFS) types was detected. Activity of non-neutralized and non-heat-treated (CFSs1), non-neutralized and heat-treated (CFSs2), pH neutralized and non-heat-treated (CFSs3) against *H. pylori* strains was evaluated. Inhibition zone test for *H. pylori* was performed using *L. reuteri* strains. lactic acid produced by *L. reuteri* was neutralized.

### RESULTS AND DISCUSSION

An agar-well diffusion method was used to assess CFS effect on *H. pylori* growth. The inhibition zones of high concentrations of CFSs were more obvious, indicating that CFS had the potential ability to inhibit *H. pylori* growth. According to results, the agar-well diffusion test showed significant inhibitory effects of *L. reuteri* against *H. Pylori*. Formation of inhibition zone was significantly due to presence of antimicrobial metabolites (e.g. bacteriocin) not secretive acid lactic.

### CONCLUSION

In summary, based on these results it can be found that the *L. reuteri* which is a probiotic can be applied in therapeutic application for treatment of disease related to *H. pylori* in gastrointestinal tract. Bacteriocin-like inhibitory substance activity can be an advantage for the probiotic choice for *H. pylori* infection control. If bacteriocin produced by *L. reuteri* has antimicrobial properties against *Helicobacter pylori*, it can be considered as a new generation of probiotics, postbiotics.

**Keywords:** *L. reuteri*, *H. pylori*, bacteriocin, agar-well diffusion, postbiotic

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Serum evaluation of enterotoxemia multivalent nanotoxoid vaccine in target animal

Mehrdad Shamsaddini Bafti, Mojtaba Alimolae, Maryam Amini, Sima Pooyan, Majid Ezatkhah, Sadegh Afzali, Amir Asadabadi Safat, Ali reza Karimabadi, Shirin Soltani

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#### ABSTRACT

The role of technology in vaccine development and research is gradually increasing. The use of nanoparticles in vaccine formulations not only increases the stability and immunogenicity of antigens but also targets the arrival of the vaccine to the effect area. Clostridium bacteria are pathogenic due to the secretion of toxins. The purpose of this project is to evaluate the serum of the multivalent nanotoxoid vaccine in the target animal based on British and European pharmacopeias. For this purpose, beta ( $\beta$ NTD-) and epsilon ( $\epsilon$ NTD-) nanotoxins of the vaccinal strains of Clostridium perfringens types B (CN228) and (CN409) D and alpha nanotoxin (NTD- $\alpha$ ) of the vaccinal strain (CN913) of Clostridium septicum after confirming the test Quality control methods based on nanoparticle concentration were used to prepare multivalent nanotoxoid vaccine formulation. According to the vaccination program, the candidate vaccine was injected into different groups of sheep. The positive control group received the quadruple clostridial enterotoxemia vaccine from Razi Institute. Indirect ELISA method was used to evaluate the antibody titer. Antibody response measurement showed high stimulation of immunogenicity and stable increase of antibody titer in groups immunized with candidate vaccine, vaccine coated with red cell membrane. The results of the study showed that sheep vaccinated with nanoparticles coated with red cell membranes show higher protective immunity (P-Value < 0.05). And the red cell membranes provide a safe layer to inhibit the nanotoxin compared to uncoated nanotoxins that cause the slow and gradual release of antigens. By using the membrane of red blood cells, the virulence of the toxin can be neutralized through the spontaneous trapping of particles in these membranes with high efficiency. The reaction of the toxin and the nanoparticle leads to the creation of a new form of nanoparticles that lose their lethality due to the involvement of the toxin in it, and these nanoparticles can easily be separated from the cell membrane and placed in the intercellular spaces due to their new shape. Stimulate the immune system.

**Keywords:** Nano toxoid vaccine, Clostridium, Enterotoxemia

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Effect of freezing temperature before freeze-dryer on the survival of *Lactobacillus plantarum*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Although freeze-drying is an excellent method for preserving microorganisms, it inevitably reduces cell activity and function to some extent, as with other driers. In addition, probiotic strains differ in their sensitivity to the freeze-drying process. Therefore, it is necessary to check the parameters related to this process. The freezing temperature before freeze-drying is an important parameter in the freeze-drying process, but it is not known whether the optimal freezing temperature before freeze-drying affects survivability of probiotics.

### MATERIALS AND METHODS

In this study, the bacterium *Lactobacillus plantarum* PTCC 1058, obtained from the Iranian Research Organization for Science and Technology, was used, and the effects of two different freezing temperatures -80 and -20°C, before freeze-drying, were investigated on the survival rate of different strains of *Lactobacillus plantarum* after freeze drying in the presence of Carriers of malt extract and maltodextrin.

### RESULTS AND DISCUSSION

According to this study, freezing at -80°C before the freeze-dryer showed a higher survival rate due to faster freezing and preventing the formation of more ice crystals in the bacterial cells. Similar studies have shown that pre-freezing temperature affects viability through changes in cell membrane integrity, membrane permeability and lactate dehydrogenase activity.

### CONCLUSION

In summary, the pre-freezing temperature is an important factor in the survival of *L. plantarum*, and the survival of this bacterium is due to a lower probability of membrane damage due to the absence of intracellular ice crystals in the faster freezing that occurs at -80 degrees.

**Keywords:** *Lactobacillus plantarum*, pre-freezing temperature, freeze-drying, survival.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Histopathological investigation in the tissues of mice following the injection of alpha *Clostridium perfringens* type A toxin

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Clostridium perfringens* (*C. perfringens*) is one of the most pathogenic species in the genus *Clostridium* that produces many toxins. This bacterium is divided into different types (A to G) based on the main toxins secreted. *C. perfringens* type A causes the destruction and damage of the cell membrane due to its ability to secrete alpha toxin, followed by vascular, muscular, and hemolytic damage as intestinal bleeding syndrome, acute intestinal inflammation, enterotoxemia, and sudden death in ruminants.

#### MATERIALS AND METHODS

At first, different strains of *C. perfringens* type A isolates were cultured in nutrient media. In order to reconfirm using molecular methods and performing Multiplex PCR using specific primers, the presence of the alpha gene in the samples was confirmed. Genotypic examination and minimal lethal dose (MLD) test with different dilutions of bacterial suspension supernatant were performed in mice. After necropsy and sampling, sampling was done for histopathological studies.

#### RESULTS AND DISCUSSION

The results showed that there was an infiltration of lymphocytes with degeneration in the liver tissue. Also, epithelial lesions observed in the brain and kidney tissues were observed. A significant difference was observed in the examination of histopathological results.

#### CONCLUSION

It concluded that alpha toxin can cause lesions in the tissues, which causes the subsequent pathogenesis of *C. perfringens* type A poisoning.

**Keywords:** Hemorrhagic syndrome, *Clostridium perfringens*, Toxin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Shielding Male Reproductive Failure in Cholestatic Rats: The Protective Potential of *Lactobacillus reuteri*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Cholestasis, a condition characterized by a reduction in bile secretion and flow, can negatively impact various organs, including the male reproductive system. *Lactobacillus reuteri* (*L. reuteri*), known for its probiotic properties and health benefits, While live probiotics have been commonly used orally, concerns about their safety in newborns and individuals with weakened immune systems have led to an interest in exploring the use of non-viable, heat-killed probiotics. Therefore, the objective of this research was to evaluate whether heat-killed *L. reuteri* can provide protection against cholestasis-induced male reproductive failure.

#### MATERIALS AND METHODS

A total of thirty-two male Wistar rats were randomly assigned to four groups, each consisting of eight rats. The first and second groups, designated as the control normal and sham control. The remaining two groups, underwent bile duct ligation (BDL) and were treated with either heat-killed *L. reuteri* (BDL + *L. reuteri*) or the vehicle (BDL-control) for a duration of 28 days. After the completion of the treatment period, the rats were anesthetized, and the levels of sexual hormones, sperm parameters and the expression of inflammatory genes were analyzed.

#### RESULTS AND DISCUSSION

In the group treated with BDL + heat-killed *L. reuteri*, the levels of sperm viability and LH exhibited a significant increase, whereas the levels of TNF- $\alpha$  and IL-6 gene expression displayed a notable decrease compared to those in the BDL group ( $p \leq 0.05$ ). Previous Studies have suggested that the immunoregulatory effects of probiotics are influenced by the presence of essential cell wall components, such as peptidoglycan, lipothoic acid, theoic acid, and exopolysaccharides.

#### CONCLUSION

Our findings suggest that heat-killed *L. reuteri* could have potential benefits in addressing male infertility.

**Keywords:** Infertility, *Lactobacillus reuteri*, Liver Fibrosis, Testis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Optimization of Culture Conditions for *Pediococcus lolii* probiotic bacterium

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Considering the diverse advantages of probiotics on public health, it is necessary to increase probiotic production and their antimicrobial effects.

Lactic acid bacteria (LAB) are well known to produce peptides and proteins with antimicrobial properties. *Pediococcus lolii* as a known LAB, has high potential to produce antimicrobial agents. In this study, we evaluated the effect of several growth parameters on the antimicrobial ability of *pediococcus lolii* bacteria isolated from traditional cheese.

#### MATERIALS AND METHODS

*Pediococcus lolii* bacteria were cultured in MRS media with different PH in the range of 4 to 6. Also, the effect of different glucose, fructose, yeast, and peptone concentrations was evaluated on the antimicrobial ability of *pediococcus lolii* by minimum inhibitory concentration (MIC) test.

#### RESULTS AND DISCUSSION

Based on the results, it was confirmed that adding 5mg/ml glucose or fructose in MRS broth has the best effect on the antimicrobial ability of *P. lolii* bacteria. Also, it was found that 5mg/ml peptone and or 2.5mg/ml yeast in pH=4 have the best effect on the growth and antimicrobial effect of *P. lolii* bacteria.

#### CONCLUSION

According to our successful findings, there is an essential need for extensive study of probiotics due to their considered role in public health.

**Keywords:** Antimicrobial Effect, *Pediococcus Lolii*, Culture Optimization

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The antibacterial effects of *Lactobacillus reuteri* isolated from yogurts against pathogenic *Helicobacter pylori*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Lactobacillus reuteri* is a well-known probiotic with beneficial effects to human health. This species can be found in different organs of the body such as skin, breast, gastrointestinal tract, and urinary tract. Due to its antimicrobial effects, the beneficial effect against *Helicobacter pylori* which is a pathogen in the human gastrointestinal tract can be tested. *H. pylori* causes infection in the stomach by attaching to gastric epithelial cells. If *H. pylori* receptors get blocked by *Lactobacillus reuteri* receptors, *H. pylori* bacteria cannot be able to attach to gastric epithelial cells and this will prevent gastric diseases. Therefore, the main aim of this study was to determine the antimicrobial properties of *L. reuteri* against *H. pylori*.

#### MATERIALS AND METHODS

To do so, different samples of yogurts around Iran were collected, then 1gr of each samples weighted and solved in 10 mL Saline, then 10 mL of this was added to 90 mL of MRS broth, following that were cultured on MRS agar and incubated to reach the colonies, each of colonies were identified and characterized by staining, biochemical and sequencing analysis to determine the species. After culturing the *H. pylori*, the co-aggregation and antibacterial effects were studied.

#### RESULTS AND DISCUSSION

The sequencing results revealed that the isolated species was *L. reuteri* and coaggregated strongly with *H. pylori* with large numbers of co-aggregation clumps formed at the bottom of the tube and a clear upper suspension. The results of co-aggregation showed that there is 91.8% co-aggregation between *L. reuteri* and *H. Pylori*. Moreover, the disc diffusion results showed significant inhibitory effects of *L. reuteri* against *H. Pylori*.

#### CONCLUSION

In summary, based on these results it can be found that the *L. reuteri* which is a probiotic can be applied in therapeutic application for treatment of disease related to *H. pylori* in gastrointestinal tract.

**Keywords:** Probiotic, Co-Aggregation, *L.Reuteri*, *H.Pylori*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Apoptosis induction of selenium nanoparticles synthesized by probiotic *Lactobacillus casei* supernatant against HT-29 colon cancer cell line

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Selenium is a trace and essential micronutrient for the health of humans, animals and microorganisms. Recently, selenium nanoparticles (SeNPs) have attracted the interest of many researchers due to their biocompatibility, bioavailability, and low toxicity. Therefore, selenium nanoparticles are widely used in various biomedical applications due to their higher bioactivity. In general, selenium nanoparticles can be synthesized by physical, chemical and biological methods. The effect of size, shape, and method of synthesis on their applications in biological systems has been investigated by many researchers.

### MATERIALS AND METHODS

In this study, the SeNPs were green synthesized using *Lactobacillus casei*. The morphology, size and stability of selenium nanoparticles were verified by characterization method. Anticancer and cytotoxic effects (on cancer cell line HT-29 and normal HFF cell) were compared, flow cytometric analysis was performed to study cell viability, cell cycle arrest and apoptosis. Changes in the expression of certain genes associated with cell cycle regulation were performed by qRT-PCR. Overexpression of caspase-3, caspase-9 and *BAX* gene by SeNPs confirmed apoptosis.

### RESULTS AND DISCUSSION

Apoptotic and antioxidant related gene expression was determined in HT-29 cells treated with Se-NPs by qRT-PCR. Overexpression of *caspase-3*, *caspase-9* and *BAX* gene by SeNPs confirmed apoptosis. SeNPs could also prevent migration and invasion of HT-29 cancer cells.

### CONCLUSION

In conclusion, the SeNPs provides more efficient biological applications that could make it a promising factor for cancer treatment.

**Keywords:** Green Synthesis, Selenium nanoparticles, colon cancer, HT-29, Apoptosis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Green synthesis of silver nanoparticles by garlic leaf extract and evaluation of its antimicrobial properties

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nanobiotechnology advancements have resulted in intriguing discoveries in science. Biosynthesis of economically and environmentally beneficial metal nanoparticles can now be done using this technology in the fields of agriculture, medicine and etc. plant components including leaves, roots, and fruits are used for biosynthesis applications, also known as “green synthesis”.

### MATERIALS AND METHODS

Herein we report the green synthesis of silver nanoparticles (AgNPs) completed using garlic (*Allium sativum*) leaf extract. Characterization of nanoparticles was carried out by UV-Vis spectroscopy, FESEM, MAP and EDS analysis. Antimicrobial activity of the AgNPs was evaluated against five distinct bacterial strains, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883 and *Proteus mirabilis* ATCC 43071, using well and disc diffusion method.

### RESULTS AND DISCUSSION

The UV-Vis spectroscopy showed an absorbance peak range between 390nm to 500nm which indicates the presence of AgNPs in the reaction solution. Using FESEM the size of the AgNPs was found to be remarkably small with a size range of 15nm to 17 nm, and spherical. The biosynthesized AgNPs also showed promising antimicrobial activity against the bacterial strains, *S. aureus* showed the highest sensitivity with an inhibitory zone around 14mm whereas the gram-negative strains showed less sensitivity towards the biosynthesized AgNPs.

### CONCLUSION

In this study the aerial leaves of *Allium sativum* were used, since they are considered agricultural waste so we could provide a way to turn them into profit, in doing so biosynthesis of stable spherical AgNPs was demonstrated using *A. sativum* leaf extract, where it acts as a reducing agent and was able to biosynthesize AgNPs with a high antibacterial activity which can have applications in industries and medicine.

**Keywords:** Green synthesis, Silver nanoparticles, *Allium sativum*, Antimicrobial

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The Antibacterial Effect of *Ganoderma lucidum* Ethanolic Extract on Three Food Poisoning Bacteria: *Bacillus cereus*, *Staphylococcus aureus*, and *Salmonella typhimurium*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Ganoderma lucidum* has been used in Asian countries to improve health and increase lifespan. The combination of triterpenoids and polysaccharides makes this mushroom a valuable nutraceutical and pharmacological source. Although *G. lucidum*, a potent natural fungus with medicinal traits, has antibacterial properties, its ability to control food-borne pathogens has not been studied. This study investigates the antibacterial properties of *G. lucidum* against foodborne pathogens including *Salmonella typhimurium*, *Staphylococcus aureus*, and *Bacillus cereus*.

#### MATERIALS AND METHODS

The *G. lucidum* dried fruit bodies used in this study were obtained from "Iran Ganoderma" and extraction was done by Soxhlet apparatus and the solvent (96% ethanol) was evaporated by rotary. To assess the antibacterial activity, the residues were dissolved in Dimethyl Sulfoxide (DMSO) (33 mg/ml). For every bacterial species that was examined, the turbidity was standardized to a value of 0.5 MacFarland standard scales. The inoculum volume was swabbed onto a Mueller-Hinton agar plate using a sterile cotton swab within 15 minutes. 30 microliters of the crude ethanolic macrofungal extract dissolved in DMSO were loaded into wells, on the agar plates. DMSO were used as negative control. The plates were incubated at a temperature of 37°C for 24 hours. The experiment was set up in triplicate, and the diameters of the zones of inhibition were measured in millimeters.

#### RESULTS AND DISCUSSION

Following a 24-hour incubation period at a temperature of 37°C, the ethanolic extract derived from *G. lucidum* exhibited an inhibition zone of 12 mm when tested against *B. cereus*, while a zone of 10±1 mm was seen against *S. aureus*. In a similar manner, no inhibition was seen in the case of *S. Typhimurium*. The extracts have the ability to prevent the growth of Gram-positive bacteria, but they do not possess the capability to inhibit the growth of Gram-negative bacteria.

#### CONCLUSION

In general, in addition to its significant nutritional composition, the ethanolic extract of *G. lucidum* exhibited antimicrobial activity against gram-positive bacteria that cause food poisoning and gastrointestinal illnesses. However, additional research is required to gain a more comprehensive understanding of the mechanism of action and application for prevention of food poisoning.

**Keywords:** *Ganoderma lucidum*, Antibacterial activity, Agar well diffusion method.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The growth of two types of microalgae named *Chlorella vulgaris* and *Scenedesmus sp.* under environmental conditions (IN VIVO)

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Microalgae are a diverse group of eukaryotic photosynthetic microorganisms that can grow rapidly due to their simple structure. *Chlorella vulgaris* and *Scenedesmus.sp* are two common strains in microalgae-based industries that can grow under both autotrophic and heterotrophic conditions. The value-added compounds mainly produced from microalgal biomass are worth to investigate their growth in two simulated media of saltwater and freshwater at environmental conditions. This study attempts to develop knowledge that can provide information and clues about the growth of algae in the natural environment in simulated medium to be applicable for commercial scale.

### MATERIALS AND METHODS

Microalgae were obtained from Iranian Research organization for science and technology (IROST). Cultured in 6 erlen flask including 250 cc of BG-11 and BBM during 7 days with equal cell number as mono- and mixed cultures. Erlen were inserted beside window to receive an indirect sun-light/dark condition at room temperature (averagely 25°C). Natural conditions of sunlight and at room temperature were considered to evaluate their growth within cultivation. On the third and seventh days, the samples were measured to count the number of cells, biomass measurement, to evaluate the viability of growth and treatment.

### RESULTS AND DISCUSSION

Based on the obtained results, generally speaking, mixed cultures of *C.vulgaris* and *Scenedesmous sp.* have shown better performance rather than their mono cultivation in terms of biomass production, increasing cell numbers and OD, and TDS reduction in both BBM and BG-11 medium.

### CONCLUSION

The results showed that both types of microalgae in both Salt and freshwater had significant cell growth. In general, the mix of microalgae in both medium cultures (BBM and BG11) had higher cell growth and biomass production

**Keywords:** BBM, BG11, Biomass, Microalgae, Salty and fresh water

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Optimizing Skim Milk medium for production of postbiotic cocktail with high antioxidant activity obtained from *Lactobacillus spp*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Probiotics, along with their postbiotic metabolites, have beneficial effects on human health. Adopting a healthy lifestyle which includes natural antioxidants such as postbiotics, can prevent oxidative stress. In the industrial sector, the use of nutritional mediums at specific concentrations in combination with prebiotics (essential elements which fermented by live cells of bacteria) is required to produce -potential postbiotics, such as those with antioxidant activity. The objective of this investigation was to enhance the antioxidant capacity of postbiotics by optimizing skim milk medium in combination with sucrose (prebiotic).

### MATERIAL AND METHODS:

In this investigation, four concentrations of skim milk (2%, 3%, 5%, and 10%) were produced, and each concentration was divided into four sections. Subsequently, four concentrations of sucrose (0.5%, 1.5%, 2.5%, and 10%) were separately added to each section. To prepare the postbiotic cocktail, initially, 10 *Lactobacillus* strains, consisting of *L. casei* 375, *L. casei* 441, *L. brevis* 165, *L. brevis* 206, *L. reuteri* 117, *L. reuteri* 118, *L. fermentum* 60, *L. rhamnusius* 66, *L. plantarum* 469, and *L. plantarum* 266 which obtained from healthy human feces were cultured in MRS medium (at 37°C for 18 hours). Then, the mixture of 10 strains with specific concentration (10<sup>9</sup> cfu/ml) inoculated in specific prepared skim milk medias and incubated for 96 h at 37°C in aerobic condition. Following bacterial growth, the postbiotic cocktail was obtained by centrifugation and filtration. To evaluate the antioxidant activity of postbiotic cocktail, biochemical tests, including DPPH, ABTS, HRS, superoxide onion, lipid peroxidation, and reducing power, were employed.

### RESULTS

Based on the findings, it was observed that there was an increase in antioxidant potential with an elevation of concentration of skim milk and decrease in concentrations of sucrose. Thus the postbiotic mixture demonstrated heightened antioxidant potential in all biochemical assessments conducted in a skim milk substrate with a concentration of 5%, accompanied by 0.5% sucrose.

### CONCLUSION

The findings have demonstrated that by means of refining the nutrient medium, the efficacy of the postbiotic mixture can be elevated, rendering it a viable candidate for employment in a diverse array of industries such as the dairy, pharmacological and clinical sectors.

**Keyword: Antioxidant activity, postbiotic, skim milk, sucrose**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Bioremediation of two simulated media of salty- and fresh- water using *Chlorella vulgaris* and *Scenedesmus* sp.

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Chlorella vulgaris* and *Scenedesmus* sp. are two major microalgae which have been used for water and wastewater treatment in recent years. Dissolved ions such as NO<sub>3</sub>, NO<sub>2</sub> and also toxic metal which make major problems for human life can be assimilated by them and meet the standard levels of treated water by microalgae growth. This study evaluates the reduction of total dissolved solids (TDS) along with biomass production in two simulated media of BBM and BG11 to predict their ability in a salt- and freshwater treatment for industrial application.

### MATERIALS AND METHODS

*C. vulgaris* and *Scenedesmus* sp. were obtained from Iranian Research Organization for Science and Technology (IROST) and cultivated in mono and mixed culture with similar inoculation cell number at room temperature for 14 days in 6 Erlenmeyer flasks (500ml) of BG11 and BBM medium under continues light condition with in intensity of 10000 lux irradiation. Counting the cell number of microalgae, pH, biomass production, optical density (OD<sub>680</sub>) and TDS and electrical conductivity were measured every day.

### RESULTS AND DISCUSSION

According to the results, mono cultivation of *C.vulgaris* and mixed cultivation of two microalgae in BBM have reduced TDS and EC rather than other cultivations. Moreover, the biomass production and optical density as well as pH have increased during 14 days when growing microalgae and assimilation of dissolved iones. It means that *C. vulgaris* as mono and mixed cultivation has ability to be applied for TDS removal in salty water medium.

### CONCLUSION

The results revealed that both microalgae species can be effective for TDS removal. In general, *C. vulgaris* has shown higher removal efficiency in most parameters in both medium cultures (BBM and BG11) than *Scenedesmus* as mono and mixed cultivation.

**Keywords:** Microalgae, TDS, BBM, BG11, Salty and fresh water

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### ***In silico* evaluation, cloning, and expression of Omp22 as a promising vaccine candidate against *Acinetobacter baumannii***

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

The prevention and control of infections still heavily rely on prophylactic strategies such as vaccination. Recently, Omp22 from *A. baumannii* has been identified as an effective vaccine candidate. However, limited data is available about this protein. This study aimed to comprehensively *in silico* analyze of Omp22 and its expression *in vitro*.

#### MATERIALS AND METHODS

The sequence of Omp22 was retrieved and then scanned for subcellular localization, antigenicity, allergenicity, homology to human proteome, physiochemical characteristics, linear and conformational B-cell epitopes, MHC binding sites, tertiary structure prediction, and molecular dockings. Additionally, the gene encoding omp22 was cloned into the pET-28a (+) vector and the expression level was optimized.

#### RESULTS AND DISCUSSION

The results showed that Omp22 has a molecular weight of 22.48 kDa, pI of 9.30, and belongs to the outer membrane proteins superfamily without transmembrane helices. Omp22 was confirmed as non-allergen with appropriate stability. Two linear B-cell epitopes were identified, as well as 108 MHC-I and 50 MHC-II binding sites, that could help to develop an epitope-based vaccine. Three conformational B-cell epitopes were identified through 3D structure prediction, and molecular docking analysis showed desirable interactions in the docked complexes. The optimized expression of the recombinant Omp22 was successfully achieved with 0.5 mM IPTG for 4h incubation at 37°C. This result can facilitate further investigations on Omp22-based subunit vaccines.

#### CONCLUSION

This study represents a significant step towards developing an Omp22-based vaccine candidate against *A. baumannii*. However, further experimental analyses are still needed.

**Keywords:** *Acinetobacter baumannii*, Omp22, Vaccine, *In silico* study, Expression

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Exploring the Potential of Anti-Inflammatory Peptides as Promising Therapeutic Agents for Ulcerative Colitis: A Systematic Review

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Ulcerative colitis (UC) remains an idiopathic disorder; nonetheless, it is likely linked to the disruption of immune responses in the intestinal lining, imbalance in gut microbial composition, and influences from environmental risk elements. Hence, there is a demand for innovative treatment approaches. Our objective is to comprehensively assess the most recent evidence regarding the use of anti-inflammatory peptides (AIPs) as a novel therapeutic strategy for inflammatory bowel disease (IBD), with a specific emphasis on ulcerative colitis.

### MATERIALS AND METHODS

This systematic study was carried out in February 2023 following PRISMA 2020 guideline. Published studies that investigate the use of anti-inflammatory peptides for UC treatment in were retrieved through searches of the literature in the Medline, Web of Science, and Cochrane databases.

### RESULTS AND DISCUSSION

Fourteen studies satisfied the predesigned criteria and were involved, in which 10 of them used animal models of UC and 4 were clinical trials in human. Results showed that H-SN1, a peptide derived from the snake's venom and glucagon-like peptide-2 dimer (GLP-2(2)), significantly inhibits TNF cytotoxicity. Moreover, oral administration of AVX-470 (bovine-derived, anti-TNF antibody) reduced TNF, MPO, and apoptosis levels in enterocytes. Maintaining gut hemostasis and reversing gut dysbiosis could be effective in UC treatment which Ac2-26 (a peptide that mimics annexin A1) were helpful in this condition. AMP-18 (gastroke-1) and MBCP (peptide derived from buffalo milk) can aid in preserving the intestinal barrier's integrity by stabilizing tight junctions (TJs). This could potentially prevent IBD from occurring.

### CONCLUSION

Anti-inflammatory peptides (AIPs) play a role in diminishing inflammation, balancing gut microbiota, and enhancing the integrity of the intestinal barrier. Nonetheless, their efficacy may be constrained by vulnerability to protease degradation or potential harm to host cells. Future investigations ought to concentrate on enhancing their pharmacokinetic attributes to maximize their therapeutic efficacy.

**Keywords:** Anti-inflammatory peptides, Ulcerative Colitis, Anti-microbial peptide, intestinal barrier's integrity, gut dysbiosis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Study on long-term preservation of micro algae spirulina platensis and Chlamydomonas reinhardtii

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#### ABSTARCT

#### BACKGROUND AND OBJECTIVES

Due to the importance of microalgae as organisms that are used in many biological processes and the production of high value materials and recombinant products, their preservation has always been considered. Microalgal strains generally are maintained in laboratories through serial subculture.

#### MATERIALS AND METHODS

In this research, some protocols have been used for the preservation of two microalgae strain, Spirulina platensis and Chlamydomonas reinhardtii. These protocols, consist of Lyophilization (Freeze-Drying), Cryopreservation at  $-20$  to  $-80^{\circ}\text{C}$  and liquid nitrogen at under  $-130^{\circ}\text{C}$ , using different concentration of DMSO (dimethylsulfoxide), Glycerol, Methanol, ethylene glycol, sucrose and sorbitol. In addition to the usual methods of subculturing in agar medium has been used.

#### RESULTS AND DISCUSSION

The results showed that the Viability of microalgae by using these methodologies is different and it is evident that the microalgae can be preserved for a long time without using expensive equipment and liquid nitrogen

#### CONCLUSION

This technique requires a lot of human power and is often time-consuming and leads to genetic change. The present study is aimed to develop an alternative method for the long-term preservation of stock cultures.

**Keywords:** Long-term preservation, microalgae, spirulina, Chlamydomonas

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Inspiring Microbial Behaviors for Innovative Solutions

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#### **ABSTRACT**

Biomimicry is an innovative approach that draws inspiration from natural biological processes and systems to solve complex problems and design novel technologies. In the realm of microbiology, biomimicry involves emulating microbial behaviors and interactions to develop new solutions and applications.

Microorganisms, due to their intricate adaptations and interactions with their environment, offer a wealth of insights for various fields. By studying how microbes perform tasks such as nutrient cycling, waste decomposition, and energy conversion, scientists and engineers can create sustainable solutions for challenges ranging from waste management to renewable energy production.

Biomimicry in microbiology has yielded remarkable results. Researchers have developed bio-inspired wastewater treatment systems that replicate the purifying actions of microbes, purging contaminants from water sources effectively and ecologically. Similarly, the study of microbial enzymes has led to the creation of environmentally friendly detergents and efficient industrial catalysts.

In medicine, understanding how microbes interact with the human body has inspired the development of probiotics and personalized microbiome therapies, enhancing digestive health and even influencing mental well-being. The intricate mechanisms that enable microbes to resist antibiotics have sparked innovative drug delivery methods, ultimately improving treatment effectiveness.

Furthermore, biomimicry extends beyond direct applications to fostering a deeper understanding of microbial ecosystems and their complex interdependencies. This knowledge aids in developing strategies for conserving biodiversity, preventing disease outbreaks, and optimizing agricultural practices.

In conclusion, biomimicry within the realm of microbiology leverages the remarkable adaptations and functionalities of microorganisms to address multifaceted challenges across various sectors. By mimicking microbial processes, scientists and engineers can create sustainable technologies that not only provide effective solutions but also promote harmony between human innovation and the natural world.

**Keywords: Biomimicry, Inspired form nature, Microbiology**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# A Review of human and dog gut microbiome similarities as a new interdisciplinary research approach

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The gut microbiome includes a set of bacteria, viruses, fungi, and other microorganisms that exist in the gut, and they have a significant impact on food digestion, strengthening the immune system, reducing inflammation, and overall health. The species and genome of the dog gut microbiome have many similarities with the human gut microbiome; Awareness of these similarities can lead to the creation of new approaches to human and dog health. This study aims to review the similarities in human and dog intestinal microbiomes as an interdisciplinary approach.

### MATERIALS AND METHODS

The desired information in this study, by searching indexed articles in GoogleScholar, Science Direct, Pubmed, SID, Magiran, Civilica, and Iranmedex, and also a free search in Google with the keywords; “Gut Microbiome”, “Human” and “Dog” has been obtained.

### RESULTS AND DISCUSSION

The common gut microbiome of humans and dogs are more similar in structure and function. Many bacterial species, including Lactobacillus, and Bacteroides, are similar in the human and dog microbiome; Also, according to studies, human and dog microbiomes have similar gene content. The reason for the similarity of the intestinal microbiome of dogs and humans is that humans and dogs live in a common situation, also, the food that humans and dogs consume is similar, and this makes the bacterial flora in their intestines similar. In addition, the intestines of humans and dogs have many similarities, which makes the bacteria in them similar.

### CONCLUSION

Having a common gut microbiome in humans and dogs can provide information about nutrition, gut health, and even diseases shared by humans and dogs. By studying the common microbiome, we can better understand how the nutrition and living conditions of humans and animals cause changes in the gut bacterial population and how these changes can affect gut health and overall health. By studying the common microbiome, one can look for new solutions to treat some diseases, including inflammatory bowel diseases, diabetes, obesity, and mental disorders in humans, and to treat skin diseases, allergies, and digestive disorders in dogs. Veterinarians and physicians can do joint and interdisciplinary scientific work on the common microbiome between humans and dogs, especially in providing good interdisciplinary colleagues' diets.

**Keywords:** Gut Microbiome, Human, Dog

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Immunoinformatics study on the 3-chymotrypsin like protease (3CL<sup>Pro</sup>) of SARS-CoV-2 as a putative antiviral agent

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Like other coronaviruses, the genome of SARS-CoV-2 is made from at least 10 open-reading frames (ORFs) which encode 2 polyproteins (i.e. pp1a and pp1ab containing 16 non-structural proteins [nsp]) and accessory proteins. The maturation of these polyproteins is occurred by an auto-proteolytic process mediated by papain-like protease (PL<sup>Pro</sup>/Nsp3) and 3-Chymotrypsin like protease (3CL<sup>Pro</sup>/M<sup>Pro</sup>/Nsp5). Among this, the 3CL<sup>Pro</sup> is considered as the SARS-CoV-2 main protease (M<sup>Pro</sup>) and becomes an attractive candidate for development anti-SARS-CoV-2 drug candidates. Accordingly, the immunoinformatics properties of SARS-CoV-2 3CL<sup>Pro</sup> were investigated to introduce a potential antiviral agent in the current study.

### MATERIALS AND METHODS

To perform immunoinformatics study on the SARS-CoV-2 3CL<sup>Pro</sup> as its main protease, its crystal structure was retrieved from the PDB database (PDB code: 7WQA). Thereafter, this structure was refined to strip the possible heteroatoms and cryopreservative agents. The refined 3CL<sup>Pro</sup> sequence was analyzed using various web-servers to predict possible linear B-Cell epitopes (BCEs). More investigations are being done to get a comprehensive immunoinformatics study and design an efficient anti-SARS-CoV-2 vaccine.

### RESULTS AND DISCUSSION

According to the results, Bepipred, Kolaskar & Tongaonkar Antigenicity, Ellipro, ABCPred, SVMtrip, and CBTope algorithms and servers predicted 9, 11, 6, 21, 3, and 14 epitopes in the 3CL<sup>Pro</sup>, respectively. This indicates the potential of 3CL<sup>Pro</sup> to elicit host immune system, including the humoral and cellular immune responses. Therefore, more immunoinformatics studies are being performed to predict MHC-I and MHC-II binding epitopes and the other properties.

### CONCLUSION

Given that SARS-CoV-2 has a substantially higher mortality rate than other coronaviruses, especially in elderly patients, many diagnostic and therapeutic agents have been introduced to combat this virus. During viral replication, the viral polyproteins (i.e. pp1a and pp1ab) synthesized using the host cell translational machinery, are processed by the 3CL<sup>Pro</sup> and PL<sup>Pro</sup> to generate an active viral replication complex. Hence, these proteases present attractive targets for small molecule inhibitors. Among this, with reference to the high conservancy of 3CL<sup>Pro</sup> among the 12 different coronaviruses, it has been considered as a good candidate to design pan-inhibitors of viral proteases, especially the SARS-CoV-2 main protease.

**Keywords:** SARS-CoV-2, Immunoinformatics, 3CL<sup>Pro</sup>, M<sup>Pro</sup>, Vaccine design, Antivirals

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Biosorption capability of Copper Acetate by *Shinella zoogloeoid* Bacteria

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

One of the applications of biosorption processes is the immobilization of microbial cells for the removal of toxic pollutants from industrial wastewater. *Shinella zoogloeoid* bacteria can degrade certain organic pollutants and also serve as a potential biosorbent for some heavy metals. Biosorption is defined as the passive uptake of metals by biomass, offering a cost-effective solution for biological purification. The aim of this study is to investigate and enhance the biosorption of copper acetate by *Shinella zoogloeoid* bacteria by altering some parameters such as pH, temperature, and incubation time.

### MATERIALS AND METHODS

In this study, the bacterial strain *Shinella zoogloeoid* DSM287 was utilized. Bacteria were cultured under non-heavy metal conditions to ensure an adequate supply of the bacterial species. The bacteria were then exposed to copper acetate with various parameters, including incubation time, pH, temperatures, and copper concentrations. Minimum inhibitory concentration (MIC) was determined to assess the bacteria's tolerance to various copper acetate concentrations. Subsequently, the copper uptake by bacteria was investigated under different conditions, including initial copper concentration, incubation time, pH, and temperature. The measurement of copper acetate content in each sample was performed using inductively coupled plasma mass spectrometry (ICP-MS).

### RESULTS AND DISCUSSION

The study results indicated that the MIC for copper acetate was found to be 700 micrograms. Moreover, the average biosorption of copper acetate by the bacteria was higher at pH 8 and a temperature of 30°C. Additionally, this process was time-dependent, with a higher uptake observed after 48 hours compared to 24 hours.

**Keywords:** Optimization, *Shinella zoogloeoid*, Copper, Biosorption

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Determining effects of nitrate, arginine, and ferrous on antibiotic recalcitrance of clinical strains of *Pseudomonas aeruginosa* in biofilm-inspired alginate encapsulates

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Biofilms play a role in recalcitrance and treatability of bacterial infections, but majority of known antibiotic resistance mechanisms are biofilm-independent. Biofilms of *Pseudomonas aeruginosa*, especially in cystic fibrosis patients infected with the alginate producing strains in their lungs are hard to treat. Changes in growth-related bacterial metabolism in biofilm affect their antibiotic recalcitrance which could be considered for new therapies designed based on these changes. In this study, effects of nitrate, arginine, and ferrous were investigated on antibiotic recalcitrance in alginate-encapsulated *P. aeruginosa* strains isolated from cystic fibrosis patients in the presence of amikacin, tobramycin, and ciprofloxacin. Also, expression of an efflux pump gene, *mexY*, was analyzed on selected strains in the presence of amikacin and ferrous.

### MATERIALS AND METHODS

Clinical *P. aeruginosa* strains were isolated from cystic fibrosis patients and minimum inhibitory concentration of amikacin, tobramycin, and ciprofloxacin was determined against all the strains. For each antibiotic, a susceptible and a resistant or an intermediate-resistant strain were selected, encapsulated into alginate beads, and subjected to minimal biofilm eradication concentration (MBEC) test. After determining MBECs, sub-MBEC concentrations (antibiotics at concentrations one level below the determined MBEC) for each antibiotic were selected and used to study the effects of nitrate, arginine, and ferrous on antibiotic recalcitrance of encapsulated strains. Effects of ferrous and amikacin on expression of the efflux pump gene, *mexY*, was studied on amikacin sensitive and intermediate-resistant strains. One-way ANOVA and t test were used as the statistical tests.

### RESULTS AND DISCUSSION

According to the results, the supplements had a dose-related effect on decreasing the number of viable cells; maximal effect was noted with ferrous, as ferrous supplementation significantly increased biofilm susceptibility to both ciprofloxacin and amikacin in all strains, and to tobramycin in a resistant strain. Also, treating an amikacin-intermediate strain with amikacin increased the expression of *mexY* gene, which has a role in *P. aeruginosa* antibiotic recalcitrance, while treating the same strain with ferrous and amikacin significantly decreased the expression of *mexY* gene, which was a promising result.

### CONCLUSION

Our results support the possibility of using ferrous and arginine as an adjuvant to enhance the efficacy of conventional antimicrobial therapy of *P. aeruginosa* infections.

**Keywords:** Antibiotic resistance, Arginine, Biofilm, Ferrous, *Pseudomonas aeruginosa*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Co-expression analysis for relative changes of LncRNA *LinP1* and genes involved in the double-stranded DNA breaks repair pathway in *Helicobacter pylori*-infected and non-infected patients with gastritis

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

Chronic gastritis is a primary inflammatory disease of gastric mucosa, which, if left untreated, can develop severe gastritis, metaplasia, dysplasia, and cancer. The annual risk of progression of chronic gastritis from one stage to the next is estimated at an average of 2-3%. *Helicobacter pylori* is a gram-negative bacterium that colonizes the stomach of almost half of the world's population and is the most common cause of gastritis (either acute or chronic) worldwide. It is estimated that about 50% of patients with *Helicobacter pylori*-associated chronic gastritis will suffer some degree of atrophic gastritis during their lifetime, and about 5% of the infected people will progress to atrophic gastritis and advanced stage of disease (stomach cancer). While the virulence factors of this bacterium are known as the main contributors to the progression of gastritis, the exact mechanism of carcinogenesis of this bacterium following the occurrence of chronic gastritis, and the induction of inflammatory responses, oxidative stress, DNA damage, and genomic instability is not yet perfectly clear. In people with *Helicobacter pylori*-associated gastritis, the activation of the error-prone DNA repair pathway (non-homologous end joining) can play a major role in the development of precancerous lesions.

##### MATERIALS AND METHODS

In this study, after obtaining informed consent from about 200 patients with chronic gastritis, biopsy samples were collected and questionnaires were filled. Patients were divided into two groups according to the results of the rapid urease test and microscopic examination. The case group included patients with chronic gastritis and *Helicobacter pylori* positive test while the control group consisted of patients suffering from chronic gastritis and negative for *Helicobacter pylori* infection. In order to measure the relative changes in the expression levels of genes involved in the non-homologous end-joining pathway, RNA was extracted from the samples, and cDNA was synthesized. Suitable primers were designed for the measurement of the expression level of each gene and the efficiency of each primer was evaluated separately. The expression level of each gene was measured by the q-Real time PCR method and the significance of the changes was determined based on statistical calculations.

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### RESULTS AND DISCUSSION

The comparison of the two study groups revealed that the expression level of *TP53* gene was significantly increased in case samples ( $p$  value= 0.009). *Lig4* and *XRCC6* genes were relatively increased in the case group, while two genes *XRCC4* and *XRCC5* and lncRNA *LINP1* showed a relative decrease in expression in the same study group. The correlation analysis of the expression of the target genes showed a significant relationship between the transcript levels of *LINP1* and *XRCC4*, *XRCC5* and *Lig4* in the case group. Furthermore, the evaluation of gene expression changes in principal component analysis (PCA) showed that patients with *Helicobacter pylori* infection have more concentrated PC scores than non-infected individuals.

### CONCLUSION

The results of the present study confirmed the role of *Helicobacter pylori* infection in causing double-stranded DNA breaks in the gastric mucosa due to the relative activation of the non-homologous end-joining repair pathway in patients with chronic gastritis compared to individuals without this infection. The significant overexpression of *TP53* and the correlation of the decrease in the expression levels of *XRCC4*, *XRCC5* and *Lig4* genes in association with *LINP1* as a regulator of non-homologous end joining pathway, confirmed the key role of *TP53* protein in preventing the accumulation of somatic mutations and progression of chronic inflammation towards cancerous lesions by blocking non-homologous end-joining pathway. Investigating other mediators affecting the non-homologous end-joining pathway, such as *MALAT1* and *ATM*, which were not studied in this paper, and identifying the regulators of the *TP53* coding gene that plays a protective role against the activation of the error-prone repair pathway (non-homologous end-joining), can introduce appropriate therapeutic methods of preventing the progression of chronic gastritis.

**Keywords:** q-Real time PCR, *TP53*, *Lig4*, *XRCC4*, *XRCC5*, *XRCC6*, *Ku70*, *Ku80*, lncRNA, *LINP1*, Non-homologous end-joining, *Helicobacter pylori*, Gastritis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Preliminary study of a bacteriocin from the halotolerant *Bacillus* DDBCC85

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Antibiotic resistance is a serious concern worldwide. Bacteriocins, from halophilic or halotolerant bacteria, can be used as new antimicrobial compounds in the pharmaceutical industry.

### MATERIALS AND METHODS

Based on previous study, DDBCC85, a gram-positive spore-forming bacillus from Bacterial Collection of Semnan University was investigated for antibacterial properties by disk diffusion method. The strain was cultured in nutrient broth and Luria-Bertani (1% glucose) media containing 5%, 7.5%, 10%, 15%, and 20% NaCl. The activity of antibacterial agent, bacteriocin, was tested against *Salmonella typhimurium*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The bacteriocin was dialyzed against 50 mM Tris buffer (pH 7) for 20 min followed by evaporation at RT. The stability of bacteriocin was examined at 4°C, -20°C, 60°C, and 100°C and pH 4, 7, and 9. The molecular weight of purified bacteriocin, BAC85, was determined using 12% SDS-PAGE gel.

### RESULTS AND DISCUSSION

DDBCC85 grew in 5-10% NaCl. An activity of 208.712 AU/ML and 39.477 AU/ML for BAC85 against *B. subtilis* obtained from 1-3, and 4 -5 days incubation of DDBCC85. Same activity was found at 37°C and 100°C while -20°C decreased the activity to 101.025 AU/ML. An activity of 148.194 AU/ML obtained for DDBCC85 incubated in 4°C and 60°C. The highest activity (208.712 AU/ML) was at pH 7. pH 4 and pH 9 decreased the activity of BAC85 to 148.194 AU/ML. BAC85 with a MW of about 30 KD showed no effect on *S. typhimurium*, *E. coli*, *S. aureus* and *P. aeruginosa*. In 2021, 94 halotolerant Bacilli were evaluated for antimicrobial and anti-biofilm activities against Bacillus strains. Furthermore, bacteriocins from Bacilli are antibiotics alternative because of their impacts on multi-drug resistants and low cytotoxicity against human cells.

### CONCLUSION

DDBCC85 grow in media containing 5-10% NaCl. The highest activity of BAC85 was against *B. subtilis* at 1-3 days, 37°C and pH=7. BAC85 from halotolerant Bacillus is a new antibacterial compound.

**Keywords: Antibacterial compound, Bacillus, Dasht Desert, Halotolerant**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Exploring the Synergistic Effect of Human Amniotic Epithelial Stem Cell-Conditioned Medium and Carboplatin on Cervical Cancer Cells: Implications for Combinatorial Therapy

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Cervical cancer is the fourth most common cancer in women worldwide, and is commonly treated with chemotherapy. However, drug resistance and adverse side effects limit the effectiveness of chemotherapy, highlighting the need for alternative or complementary therapies. In this study, we examined the potential of conditioned medium derived from human amniotic epithelial stem cells (hAEC-CM) as an adjunct therapy to carboplatin in treating cervical cancer.

### MATERIALS AND METHODS

Human term placentas were gained from uncomplicated Cesarean sections from healthy donor women. The amnion was peeled from the chorion, and the epithelial stem cells were isolated, cultured, and their conditioned medium was collected. First, the effect of hAEC-CM on cervical cancer cells was evaluated. Then, the cell growth inhibition effect of different concentrations of carboplatin on HeLa cervical cancer cells was examined, and subsequently the combination of hAEC-CM and carboplatin tested. The MTT assay was used to detect the cell viability of cells treated with carboplatin, hAEC-CM, and in the combination group.

### RESULTS AND DISCUSSION

Our results showed that hAEC-CM alone had a moderate inhibitory effect on HeLa cells, while carboplatin alone had a dose-dependent cytotoxic effect. Notably, the combination of hAEC-CM and carboplatin led to a significant reduction in the IC<sub>50</sub> of carboplatin, indicating a potential synergistic effect.

### CONCLUSION

The findings suggest the potential of hAEC-CM as a complementary therapy for carboplatin in the treatment of cervical cancer. Further studies are needed to elucidate the underlying mechanisms of this synergistic effect and to explore the clinical potential of this combination therapy.

**Keywords:** Cervical Cancer, human amniotic epithelial stem cells, hAEC-CM, stem cells, Carboplatin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of Cutaneous Manifestations in COVID-19 Patients and Their Relationship with Disease Severity

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

At the beginning of COVID-19 pandemic, highly accurate information about the clinical manifestations of the disease was not available, and the reported symptoms were non-specific and more related to respiratory symptoms such as fever, dry cough, fatigue, and sputum production. As time has passed, skin manifestations have been proposed as one of the clinical manifestations of COVID-19 in some patients.

### MATERIALS AND METHODS

In the current review, the skin findings of patients in association with COVID-19 were summarized into the categories of maculopapular or morbilliform lesions, urticarial lesions, chilblain-like lesions, vesicular lesions, petechiae or purpura lesions, and livedoid lesions.

### RESULTS AND DISCUSSION

Among all reported lesions, livedoid lesions appeared simultaneously with the symptoms of SARS-CoV-2, mainly in elderly people with severe infections, and were associated with the highest risk of the mortality of all skin lesions.

### CONCLUSION

Knowledge of the skin manifestations that may be the only symptoms of COVID-19 may help in early diagnosis and specific treatment.

**Keywords:** COVID-19, SARS-CoV-2, Cutaneous manifestations, Skin, Disease severity

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Study of antibiotic resistance and anti-biofilm formation of *Pseudomonas aeruginosa* isolated from clinical samples in Kerman hospitals, Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa* is an opportunistic pathogen in the hospital environment that associated with several human infections. Treatment of infections caused by *P. aeruginosa* is one of the biggest medical problems due to the formation of biofilm and widespread antibiotic resistance. Accordingly, the aim of this research was to investigate antibiotic resistance and anti-biofilm formation of *Pseudomonas* strains collected from hospitalized in Kerman, Iran in 2023.

#### MATERIALS AND METHODS

In this project, 30 isolates of *Pseudomonas aeruginosa* were isolated from clinical samples in Kerman hospitals. Biochemical tests were used for bacteria identification. The antibiotic resistance was determined by Kirby-Bauer disk diffusion susceptibility test. Minimum inhibitory concentration by gentamicin was done with broth micro-dilution method. Hydrophobicity of the bacteria was investigated by xylene for biofilm analysis. Biofilm formation was examined on the surfaces of glass and plastic under static and shaking conditions. Anti-biofilm formation was evaluated using  $\frac{1}{2}$  and  $\frac{1}{4}$  MIC of gentamicin.

#### RESULTS AND DISCUSSION

Results showed sensitivity to gentamicin (70%), ciprofloxacin (60%), ofloxacin (56.6%), amikacin (53.35), trimethoprim sulfamethoxazol (36.6%), imipenem (30%), ceftizoxime (3.3%). Minimum inhibitory concentration of the gentamicin was observed in concentration of 64 micrograms/ml (20%). Three bacteria with hydrophobicity above 70% and three bacteria with hydrophobicity below 30% were selected for biofilm tests. Biofilm production on plastic was more than glass surface, also in shaking state was more than stationary. Quantity of total bacteria was reduced from 8000 to 4400 in  $\frac{1}{4}$  MIC and 3610 in  $\frac{1}{2}$  MIC of gentamicin on biofilm formation of venous catheter.

#### CONCLUSION

According to the use of plastic medical devices such as angiocaths and venous catheters and the high ability of *Pseudomonas aeruginosa* to create biofilm on plastic surfaces, the possibility of hospital infections with *Pseudomonas* is very high and the indiscriminate use antibiotics increases their resistance. These results should be considered to understand the importance of limiting antibiotics and new health care.

**Keywords:** *Pseudomonas aeruginosa*, Antibiotic resistance, Hydrophobicity, Anti-biofilm

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Inhibitory effect of Thyme and Citrus honeys on the biofilm formation of a multi drug-resistant *Pseudomonas aeruginosa* isolate

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa* is an opportunistic human pathogen with high ability to form antibiotic-resistant biofilms. Honey has been used since ancient times for its nutritional and medicinal properties, especially for its antimicrobial activity. In this study the effect of Thyme and Citrus honeys on the biofilm formation of a resistant strain of *P. aeruginosa* was investigated.

#### MATERIALS AND METHODS

A multi drug-resistant strain of *P. aeruginosa* which was isolated from the urinary tract of a patient referred to Imam Reza Hospital was used in this study. The primary assay by disc diffusion method showed resistance of this bacterium against cefazolin, ceftizoxime, ampicillin, ciprofloxacin, nitrofurantoin, cefepime, and clavulanic acid. The effect of different concentrations (100-70% v/w) of Thyme honey as well as Citrus honey on bacterial growth and its biofilm formation capacity was assessed by the well-diffusion and microtiter-plate methods, respectively.

#### RESULTS AND DISCUSSION

The result obtained from the well-diffusion method showed that 100% concentration of Thyme and Citrus honeys inhibited the growth of multi drug-resistant *P. aeruginosa* with inhibition zone diameters of 15 and 20 mm, respectively. Statistical analysis results obtained from the microplate reader showed that all the concentrations of both honeys show the inhibition effect on biofilm formation of recruited *P. aeruginosa* isolate.

#### CONCLUSION

The study revealed antibacterial and antibiofilm activities of Thyme and Citrus honeys against multidrug-resistant *P. aeruginosa* which can cause devastating acute and chronic infections in individuals with compromised immune systems. This result suggests that these honeys might find a place in clinical practice, alone or as part of combination antimicrobial therapies, to treat multidrug-resistant bacteria.

**Keywords:** Honey, *Pseudomonas aeruginosa*, Biofilm

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# A new perspective on the use of probiotic bacteria and plant extracts on the reduction of oxalate kidney stones

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### ABSTRACT

Hyperoxaluria is the major risk factor for the formation of Calcium oxalate stones. The purpose of this study was to evaluate the *in vitro* effect of the simultaneous administration of oxalate-degrading bacterias, *Tribulus Terrestris*, *Urtica dioica*, and *Zea mays* extract in reducing urinary oxalate. 24 Male Wistar rats were randomly divided into 4 equal groups (n=6). The rats of group I received a normal diet (positive control group) and groups II (negative control group), III, IV rats received a diet containing ethylene glycol (3%v/v) for 30 days. Groups III rats received *Zeamays*, *Urtica dioica*, and *Tribulus Terrestris* extract. Group IV rats received plant extracts and probiotics in combination for 30 days. Also, 4 strains of *Lactobacillus*, 2 strains of *Bifidobacterium* and 2 strains of *L.paracasei* (that showed capability in oxalate degrading in culture media) were used as probiotics. 24-hours urine samples were collected on day 0, 15, and 30. Urine volume, oxalate, calcium, and creatinine levels were measured. Animals were sacrificed and kidneys were harvested, weighed and histopathologically evaluated for calcium oxalate crystals. Finally, the plant extracts were analyzed by GC-Mass. The obtained results Showed that EG significantly increased urine oxalate, calcium, and creatinine levels, and CaOx deposits. Provision of extracts and probiotics combination resulted in significantly lower levels of urine oxalate, calcium, creatinine and CaOx depositions as compared to Group II. The use of herbal extracts with Probiotic can be effective in the prevention and treatment of urolithiasis.

**Keywords:** Calcium oxalate crystals, GC-Mass, plant extracts, Probiotic,

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Antibacterial activity of postbiotics metabolites against *Mycobacterium avium* subsp. *paratuberculosis*: an in vitro study

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) is a slow-growing bacterium that primarily affects ruminants, causing a chronic disease known as Johne's disease and Crohn's disease in human. Despite extensive research efforts, effective control strategies for this infection remain elusive due to bacterium's ability to evade host immune responses and its resistance to conventional antimicrobial agents. Postbiotics are bioactive compounds produced by lactic acid bacteria during the fermentation process. Postbiotics exhibit antimicrobial properties against pathogenic bacteria and can potentially be utilized as natural alternatives to antibiotics. The aim of this study was to investigate the antibacterial effect of postbiotics derived from *Lactobacillus casei* and *Lactobacillus plantarum* in vitro.

##### MATERIALS AND METHODS

The broth susceptibility testing was done using mixed commercialized postbiotics in the Middlebrook 7H9 broth. The final concentrations were adjusted to 50, 25, 12.5, 6.25, 3.125 mg/mL and then incubated with 100 mL of bacterial suspension (approximately to 10<sup>6</sup> CFU). The tubes were incubated at 37°C and the OD<sub>600</sub> was monitored for 42 days at regular periods and compared with no added postbiotics cultures. The all procedures were repeated at least two times and the lowest concentration of the postbiotics with no detectable bacterial growth (turbidity) was considered the MIC.

##### RESULTS AND DISCUSSION

The different concentrations of postbiotics showed the antibacterial activity against MAP. The MIC of postbiotics was 6.25 mg/mL. The probable mechanisms of postbiotics antibacterial activity are due to producing bacteriocins such as Lactocin 705 from *L. casei* and plantaricin from *L. plantarum*.

##### CONCLUSION

The results showed that postbiotics could be a practical bioactive compound for prevention and treatment of enteric pathogens such as MAP.

**Keywords:** *Mycobacterium avium* subsp. *Paratuberculosis*, postbiotics, MIC, *Lactobacillus casei*, *Lactobacillus plantarum*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the antibiotic resistance pattern of *Escherichia coli* isolates causing urinary tract infection in patients referred to Imam Khomeini Hospital in Shirvan city in the year 2022

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Urinary tract infection is one of the most common bacterial infections around the world, and *Escherichia coli* is the most common cause of urinary tract infections in the world. Considering the increasing antibiotic resistance and since there is a direct relationship between antimicrobial levels prescribed and the level of resistance seen in a wide range of organisms. Therefore, this study was conducted with the aim of investigating the antibiotic resistance of *Escherichia coli* at Imam Khomeini Hospital in Shirvan.

### MATERIALS AND METHODS

In this cross-sectional descriptive study that was conducted from April to March 2022, 2089 urine culture samples were sent from outpatients and inpatients at Imam Khomeini Hospital in Shirvan city, 122 samples were positive. Different bacterial culture environments as well as different biochemical tests were used to identify bacterial isolates and disc diffusion method was used to check the antibiotic resistance of *Escherichia coli* bacteria.

### RESULTS AND DISCUSSION

Out of 122 positive samples in 1401, 77 samples are related to *Escherichia coli* bacteria, of which ampicillin-sulbactam antibiotic (66.66%) is the most resistant and nitrofurantoin (3.22%) is the least resistant. Resistance of other antibiotics, respectively, Cefotaxime (58.9%), Cefixime (57.1%), Cefazolin (51.21%), Doxycycline (45.9%), Ceftazidime (45%), Ciprofloxacin (31%) Gentamicin (9.21%).

### CONCLUSION

In general, according to the results, the most resistant antibiotics are ampicillin-sulbactam and vesofotaxime, which are recommended not to be prescribed due to their resistance and ineffectiveness in treatment. Nitrofurantoin and gentamicin are the most sensitive, respectively.

**Keywords:** Urinary tract infection, *Escherichia coli*, antibiotic resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# CRISPR-Cas9: A Tool for Attenuating *Shigella sonnei* by Targeting the *MxiE* Gene

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## ABSTRACT

*Shigella sonnei* is a common bacterial pathogen responsible for gastrointestinal infections in humans. The development of new therapeutic strategies to combat *Shigella sonnei* infections is of great importance. In this study, we used the CRISPR-Cas9 gene editing system to target and delete the *MxiE* gene in *Shigella sonnei*. The *MxiE* gene encodes a transcriptional regulator that plays an important role in the pathogenicity of *Shigella sonnei*. By disrupting the function of this gene, we aimed to discover its precise contribution to bacterial virulence.

To achieve deletion of the *MxiE* gene, we used the CRISPR-Cas9 system, which enabled precise and efficient gene editing. We designed a guide RNA (gRNA) specific for the target site in the *MxiE* gene and introduced it into *Shigella sonnei* strains expressing Cas9 endonuclease. Also, a DNA fragment (HDR fragment) was designed to incorporate into the bacterial genome following the cleavage of the bacterial genome by Cas9 enzyme. After the gene editing process, we confirmed the successful deletion of the *MxiE* gene using PCR analysis, enzyme digestion and DNA sequencing.

The results of PCR, restriction digestion and sequencing showed the successful recombination of *S. sonnei*. We will next investigate the effect of *MxiE* gene deletion on the pathogenicity of *Shigella sonnei* through a series of in vitro and in vivo experiments. We hypothesize that a significant reduction in the ability of *MxiE* deleted strains to invade and induce cytotoxicity in host cells is observed. In the following we will develop a mouse infection model, mice challenged with *MxiE* deleted strains.

This study not only highlights the successful application of the CRISPR-Cas9 system for gene deletion in *Shigella sonnei*, but also provides valuable insights into the role of the *MxiE* gene in bacterial pathogenicity. The findings of this study contribute to a better understanding of the molecular mechanisms underlying *Shigella sonnei* infections and provide potential avenues for the development of targeted therapeutic interventions.

In conclusion, our study demonstrates the efficiency of the CRISPR-Cas9 system in deleting the *MxiE* gene in *Shigella sonnei*, leading to reduced virulence. This research lays the foundation for further research into the development of innovative treatments and preventive measures against *Shigella sonnei* infections.

**Keywords:** CRISPR-Cas9 gene editing, Gene deletion, Bacterial pathogenesis, Virulence factors

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The effect of pH and temperature on the degree of hydrolysis and antioxidant activity of *Spirulina* cell extract

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

Increasing the consumption of natural materials has increased the request for biological sources such as *Spirulina*. *Spirulina* is considered one of the healthiest foods because of its high protein content, vitamins, minerals, carotenoids, essential fatty acids antioxidants, and phycocyanin. It has high nutritional value and promising health benefits such as antioxidant, immunomodulatory, and anti-inflammatory activities that have already been explained. The aim of this study was to investigate the effects of pH and temperature on the degree of hydrolysis (DH) and potent antioxidant properties of *Spirulina* cell extract.

### MATERIALS AND METHODS

For this purpose, Zarrouk medium was used to culture *Spirulina*. *Spirulina* cells were disrupted with ultrasonication. The resulting cell extract was lyophilized. The protein value of cell extract was evaluated with the Lowry method. The degree of hydrolysis was measured by ortho-phthalaldehyde (OPA) test. The antioxidant activity was evaluated by assessing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.

### RESULTS AND DISCUSSION

Results demonstrate that the degrees of hydrolysis in different hours at pH= 8 and 37°C do not differ significantly ( $P \leq 0.05$ ). The results at pH= 8 and 55°C also showed the same results ( $P \leq 0.05$ ). There were no statistically considerable differences in DH at different times on 37°C and pH=2. Comparing the degree of hydrolysis in different conditions (pH= 2 and 37°C, pH= 8 and 37°C, pH= 8 and 55°C) showed that the highest degree of hydrolysis was obtained at pH=2 and 37°C ( $17.655 \pm 0.428$ ). The lowest DPPH radical scavenging activity was obtained at pH= 8 and 55°C, which were not significantly different from each other. The highest DPPH radical scavenging activity was obtained at pH= 2 and 37°C ( $65.483 \pm 2.521 \mu\text{M TE/mg protein}$ ). The higher degree of hydrolysis obtained at pH=2 and temperature of 37°C showed more antioxidant activity than the other conditions mentioned above.

### CONCLUSION

This study has demonstrated that pH= 2 and 37°C have caused *Spirulina* proteins are more hydrolyzed and produced bioactive peptides that have a greater ability to scavenge the DPPH radicals. It has been suggested that *Spirulina* cell extract is more reductant at pH= 2 than pH= 8 at 37°C. *Spirulina* cell extract can be used as a functional food. They can prevent various diseases and disorders.

**Keywords:** *Spirulina*, cell extract, degree of hydrolysis, antioxidant activity

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation and detection of *Brucella* species from patients suspected to brucellosis in North Khorasan, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Brucellosis, caused by various species of the genus *Brucella*, remains a significant public health concern globally. Timely and accurate diagnosis of this infectious disease is crucial for effective management and control. In this study, titled "Isolation and detection of *Brucella* species from patients suspected to brucellosis," the researchers aimed to isolate and identify *Brucella* species among patients presenting with suspected brucellosis.

### MATERIALS AND METHODS

Blood samples were obtained from patients suspected to Brucellosis. Serum was separated and used for 2me and wright test. Also, blood samples were cultured on brucella agar and incubated in 72°C for 1 week. Bio typing and phage typing tests using were performed for positive samples.

### RESULTS AND DISCUSSION

Among 200 suspected patients, 50 were serologically positive. Also, blood culture of 5 patients tested positive. Bio typing revealed that all positive samples were infected with *Brucella melitensis* biovar 1.

### CONCLUSION

The study successfully isolated and identified *Brucella* species among patients suspected to have brucellosis. The combination of serological testing, bacterial culture, and molecular methods allowed for accurate detection and characterization of the pathogen. The predominance of *Brucella melitensis* biovar 1 suggests a specific strain responsible for human brucellosis in the North Khorasan population. These findings contribute to our understanding of the epidemiology and help guide appropriate treatment and prevention strategies for brucellosis.

**Keywords:** *Brucellosis, Epidemiology, PCR*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antiproliferative activity of *Lactobacillus buchneri* probiotic bacteria in HT-29 colon cancer cells

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## ABSTRACT

Based on GLOBOCAN 2018 data, colorectal cancer (CRC) is the third most deadly and fourth most detected cancer worldwide. However, numerous modern medical methods are available for CRC treatment, the survival rates are poor with some unfavorable treatment-related consequences, which affects the quality of life. The current experimental evidence supported that probiotics and their metabolites keep the CRC patients safe from treatment-related side effects. Therefore, the current study aimed to evaluate the effect of cell free supernatant (CFS) prepared from *Lactobacillus buchneri* isolated from traditional dairy products and to investigate its cytotoxic activity and apoptosis induction characteristics on HT-29 colon cancer cell line. MTT assay and Annexin V-FITC analysis were performed to evaluate the induction of apoptosis in HT-29 treated cells. The CFS of *L. buchneri* prevented the HT-29 cancer cells growth in a dose- and time-dependent manner, which it showed the highest anticancer activity at 200 µg/mL after 72 h. The apoptotic activity of CFS was also confirmed by flow cytometry results, which showed the highest incidence of apoptosis in HT-29 cancer cells treated with IC<sub>50</sub> concentration of CFC after 72 h. The cell cycle analysis also reported that the treatment of HT-29 cancer cells with the This investigation demonstrates that CFS of *L. buchneri* obtained from Iranian traditional yogurt can protect patients from CRC development by inducing apoptosis in cancer cells, which can be employed as a protective anticancer agent in controlling CRC.

**Keywords:** Colorectal cancer, supernatant of Probiotic, *L. buchneri*, Apoptosis, Anti-cancer

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# *Coxiella burnetii* infection in pregnant and aborted women: A comprehensive systematic review and meta-analysis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Complications of Q fever infection during pregnancy have been overlooked worldwide, with health systems paying little attention to this issue. Therefore, this study aimed to provide evidence regarding the association between Q fever infection and its effects on human abortion.

### MATERIALS AND METHODS

We conducted an extensive search of English electronic databases, including Google Scholar, Medline, PubMed, Science Direct, Scopus, and Web of Science, covering the period from 1993 to 2021. The search strategy involved using specific keywords related to "*Coxiella burnetii*" or "Q fever" in conjunction with "pregnancy," "pregnant," "women," "miscarriage," "humans," or "abortion" in the title or abstract.

### RESULTS AND DISCUSSION

A comprehensive analysis of 25 research papers was conducted in this review. The overall pooled seroprevalence of *C. burnetii* was found to be 11.10% (95% CI: 6.06-13.13) among the studied population. Notably, women with a history of abortion showed a higher seropositivity rate (13.68%), which was statistically significant (OR: 1.58, 95% CI: 1.24-2.02) compared to women without a history of abortion (12.66%). Furthermore, the pooled molecular prevalence of *C. burnetii* was observed to be 0.30%. Among women with a history of acute Q fever, the detection rates using culture and PCR methods were 35.61% and 13.97%, respectively. These findings show the prevalence and associations of *C. burnetii* infection in pregnant and aborted women, emphasizing the importance of further research in this area.

### CONCLUSION

Given the risks posed by Q fever infection during pregnancy, as well as the obstetric complications associated with this disease, there is an urgent need for the health system to Q fever, particularly in regions where the disease is endemic.

**Keywords:** *Coxiella burnetii*, Q fever, Pregnancy, Abortion, Women.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### *Coxiella burnetii* in small ruminant abortion samples: A molecular study in Southeast Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Domestic animals are known as the main reservoir of *Coxiella burnetii*. This infectious disease is associated with abortions in domestic animals, resulting in substantial economic losses and posing risks to human health. Therefore, this study aims to investigate *C. burnetii* in abortion samples of small ruminants in southeastern Iran.

#### MATERIALS AND METHODS

This study was conducted in Zarand city, located in Kerman province, between 2020 and 2021. A total of 50 abomasum swab samples were collected from aborted sheep and goat fetuses and analyzed using molecular methods to detect the presence of *C. burnetii*.

#### RESULTS AND DISCUSSION

The study's findings revealed that 26% (n: 13) of the collected abortion samples were found to be infected with *C. burnetii*. Among the positive samples, two samples (50%) belonged to goat abortion samples, while 11 samples (23.9%) belonged to sheep abortion samples.

#### CONCLUSION

This study highlights *C. burnetii* as one of the causative agents of abortion in small ruminants in southeastern Iran. Given the economic impact of this pathogen on livestock and its potential implications for human health in the country, it is crucial to pay close attention to *C. burnetii* in domestic animals.

**Keywords:** *Coxiella burnetii*, Q fever, small ruminants, abortion, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Detection of *Rickettsia* genus in small ruminant and their ticks in Western of Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Tick-borne rickettsioses cause disease in humans and pose serious risks to their health. Considering the importance of this pathogen in the occurrence of infection in humans and animals, which leads to mild to severe disease and even death all over the world, and limited information is available in this regard in Iran, the aim of this study is to investigate *Rickettsia* spp. in small ruminants and their ticks in Western of Iran.

#### MATERIALS AND METHODS

In this study 250 blood samples were collected from sheep and goats, as well as 244 tick samples were collected from them in Kurdistan province (western Iran), and *Rickettsia* spp. were investigated in these samples by the use of the molecular method.

#### RESULTS AND DISCUSSION

The tick species collected during this study included *Rhipicephalus sanguineus*, *Rhipicephalus turanicus*, *Haemaphysalis concinna*, and *Dermacentor marginatus*. Among these ticks, a total of 131 specimens (53.7%) were found to be infected with *Rickettsia*. Notably, *R. slovacica* (59.2%) and *R. hoogstraalii* (16.3%) were the most prevalent species identified in this study. Additionally, other rickettsia species, including *R. raoulti*, *R. massiliae*, *R. sibirica*, and *R. conorii* subsp. *israelensis* were also identified. No positive samples were observed in the blood samples collected from small ruminants.

#### CONCLUSION

The findings of this study emphasize the severity of rickettsial infection in ticks in western Iran. Due to the fact that many of the species identified in this study can cause disease in humans, it requires more attention from health systems in these areas.

**Keywords:** *Rickettsia*, Tick, Rickettsiosis, Tick born disease, Iran.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating the bacteriological quality of drinking water in water coolers of contractor workshops under the supervision of Health, Safety and Environment (HSE) Department of Sistan and Baluchestan Water and Wastewater Company

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Water quality is one of the issues that is directly related to health, individual and public health of the society, and despite various efforts and modern technologies that have been used to produce healthy drinking water, water-borne diseases among the most common diseases are infectious and are still a concern. The present study was conducted with the aim of investigating the bacteriological quality of water in water coolers of contractor workshops under the supervision of Health, Safety and Environment (HSE) Department of Sistan and Baluchestan Water and Sewerage Company.

#### MATERIALS AND METHODS

The study is of a cross-sectional type and the number of 10 water coolers located in contractor workshops under the supervision of the Department of Health, Safety and Environment (HSE) of Sistan and Baluchestan Water and Sewerage Company and the values related to pH, total coliforms, endothermic coliforms (*E. coli*), heterotrophic bacteria (HPC) and residual chlorine were measured. Data analysis was done descriptively by EXCEL and SPSS software.

#### RESULTS AND DISCUSSION

The results showed that the pH values of the samples before and after cooling water were in the range of 7.3-7.6. Also, the range of residual chlorine in the water before and after entering the water cooler has been measured in the range of 0.3-0.9 ppm and 0.4-0.7 ppm, respectively. By investigating of the number of heterotrophic bacteria colonies in cultured samples before and after cooling water, the results showed that this number was in the range of 400-1200 cfu/100 ml. Also, in some of samples, the confirmation stage related to bacteria of the general form group was positive. After the monthly monitoring, the values related to the above parameters were checked and the results showed a significant decrease in the amount of bacterial contamination.

#### CONCLUSION

The results of this research showed that there is a possibility of the presence of contaminating microorganisms in the tap water network and water cooler, although in small amounts, and monthly monitoring can be effective in reducing these contaminations. Therefore, regular control and monitoring of drinking water located in workshops is very important for the purpose of water hygiene and maintaining the health of respected workers and preventing the occurrence of any epidemic caused by contaminated water.

**Keywords:** Drinking Water, Water Cooler, Quality of Bacteriology, HSE

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# Green synthesis of gold nanoparticles by *Serratia marcescens* isolated from agricultural soils

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Gold nanoparticles have various applications in biomedicine, drug delivery, etc. In most cases, gold nanoparticles are synthesized using chemical or physical methods. These often require expensive equipment and chemicals. Therefore, need for alternative, less expensive methods, for the synthesis of gold nanoparticles. Biological synthesis of gold nanoparticles has received more attention due to the advantages of cost-effectiveness and environment-friendly nature. In this research, a native strain of bacteria from the agricultural soils of Qom was evaluated for the green synthesis of gold nanoparticles.

### MATERIALS AND METHODS

The samples were collected from agricultural soils of Qom province.

The isolated strain *Serratia marcescens* was inoculated into 100 ml of MGYB broth. It was incubated in a shaker incubator (160 rpm), at 30°C for 48 hours. The culture was then centrifuged at 5000 rpm and 4°C. Supernatant solution was prepared with 50 cc of HAuCl<sub>4</sub> solution, it was mixed and then placed in shaker incubator at 30°C and 150 rpm for 96 hours. To confirm the presence of nanoparticles and determine their characteristics, respectively, from the methods of color change, UV-Vis, DLS, XRD and Electron microscope (TEM) were used.

### RESULTS AND DISCUSSION

The obtained results showed after 48 hours of exposure to HAuCl<sub>4</sub>, the supernatant changed color to purple. The UV-Vis showed this absorption maxima at 530 nm. The samples were analyzed by a DLS device. Particle size in the range of 7-60 nm. The diffraction method (XRD) proves that the synthesized particles are gold nanocrystals. Electron microscope photos showed the nanoparticles are spherical and about 60 nm in size.

### CONCLUSION

According to the results obtained from this research, the native strain of *Serratia marcescens* isolated from agricultural soils of Qom province is a suitable candidate for green synthesis of gold nanoparticles with biotechnological application in food and pharmaceutical industries.

**Keywords:** Green synthesis, Gold nanoparticles, *Serratia marcescens*, agricultural soils

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Identification of isolated lactobacillus from traditional dairy products of Maragheh

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Probiotics are microorganisms that are beneficial for health when used in sufficient quantity. Various bacterial genera most commonly used in probiotic preparations are *Lactobacillus*, *Bifidobacterium*, *Escherichia*, *Enterococcus*, *Bacillus* and *Streptococcus*.

Nowadays, the research suggests that these “healthy” bacteria have beneficial effects on gastrointestinal dysfunctions, including diarrhea, as well as the immune system and conditions such as allergy, in children, adults, and in the oral cavity.

### MATERIALS AND METHODS

To identify the isolated bacteria from traditional Maragheh yogurt, biochemical and microbial tests including; gram staining, oxidase, catalase, hemolysis, acid and bile salt resistance mobility, nitrate reduction, SIM, sugar fermentation, antibiotic resistance, antimicrobial tests were carried out. The asparaginase and glutaminase production was evaluated by raid plate assay. The molecular identification of the isolate was down through DNA extraction, PCR amplification of 16S rRNA gene, electrophoresis and construction of phylogenetic tree of the isolate via neighbor-joining method by BioEdit and MEGA softwares.

### RESULTS AND DISCUSSION

According to the results, M24 isolate showed; gram-positive morphology, negative results for catalase and oxidase tests, resistance to bile salts and acidic pH, absence of hemolysis, resistance to antibiotics, sugar fermentation and nitrate reduction. The results of molecular investigations revealed that the isolate had the highest genetic similarity (97%) to *Limosilactobacillus fermentum* CECT562(T)AJ575812. The nucleotide sequence of the M24 isolate has been recorded in the NCBI database under accession number 568204.

Furthermore, the isolate produced asparaginase and glutaminase enzymes.

### CONCLUSION

Due to the probiotic potential of the isolate, its encapsulation via biopolymers is running in our lab.

**Keywords: Probiotic, lactic acid, Maragheh yogurt**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Effective Treatment of Polymicrobial Infections from Diabetic Wound Infections in Mice using Bacteriophage and Gentamycin Combination therapy

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

: Diabetic patients are more susceptible to developing wound infections resulting in poor and delayed wound healing. Drug-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*) *Staphylococcus aureus* (*S. aureus*) are among the most frequently identified pathogens in diabetic foot ulcers (DFUs). Therefore, it seems necessary to search for alternative treatment methods such as phage therapy. Bacteriophages are viruses that target specific bacteria and can be used as a suitable alternative to antibiotics in the treatment of bacterial infections. The aim of this study was assessment of bacteriophage and gentamycin combination effects on bacterial isolates from DFU infections.

### MATERIALS AND METHODS

In this study, we assessed the effect of co-therapy using bacteriophage and gentamycin. For this, first, bacteriophage was prepared. The sewage and animal feces samples were processed and the phages were enriched using *S. aureus* and *P. aeruginosa* as host organisms. The lytic potential of phage isolates was assessed by the clarity of plaques. Moreover, we used diabetic wounds in a mouse model to study the efficacy of phage therapy in abrogating fatal *S. aureus*, *P. aeruginosa*, and the infections caused by both of them. Besides, we assessed the effect of simultaneous administration of phage and antibiotics on the infected diabetic wounds *in vivo*. We also assessed the effects of HPMC gel containing phage cocktail topically on animal models of DFU.

### RESULTS AND DISCUSSION

We isolated and characterized four lytic phages: Stp2, psp1, Stp1, and psp2. The phage cocktail was prepared and investigated *in vitro*. Results revealed that the phage cocktail significantly reduced the mortality rate in diabetic infected mice. We determined that cocktail bacteriophage treatment effectively decreased bacterial colony counts and improved wound healing in *S. aureus* and *P. aeruginosa* infections, especially when it is administered concomitantly with gentamycin.

### CONCLUSION

As a result, the use of complementary therapy using a phage cocktail together with antibiotics can be an effective method for the treatment of infected wounds.

**Keywords:** Bacteriophage, Gentamycin, Diabetic Wound Infection, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Biochemical and molecular identification of probiotic bacteria isolated from traditional dairy products of Maragheh County

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The word probiotic is derived from the Greek word probios, meaning for life. This term was first used by Stillwell and Lilley in 1965 for microorganisms that have a positive effect on the growth and balance of intestinal microbial flora. Dairy products are very favorable carriers for probiotics. Probiotics are found in dairy products such as milk, yogurt, cheese, ice cream, buttermilk, and sour cream. In addition to live bacteria, probiotic yogurts provide the host with nutrients such as biological peptides and calcium. Lactic acid bacteria are among the most important probiotics, the most famous of which are *Bifidobacterium* and *Lactobacillus* genera.

### MATERIALS AND METHODS

In this study, M09 isolate was isolated from traditional yogurt samples of Maragheh. After purification, preliminary tests were conducted to evaluate probiotic characteristics such as gram, catalase, hemolysis, oxidase, acid and bile salts resistance tests and biochemical and microbial tests including sugar fermentation, SIM, nitrate reduction, antibiotic resistance and antimicrobial. In order to the molecular identification of the isolate, DNA was extracted, 16S rRNA gene amplified by PCR, PCR result visualized using gel electrophoresis. Then, the phylogenetic tree of the isolate was constructed by neighbor-joining method via BioEdit (version 7.2) and MEGA (version 7) softwares.

### RESULTS AND DISCUSSION

According to the biochemical, phenotypic and microbial findings, the isolate was gram-positive bacteria, negative results for catalase and oxidase tests, resistance to bile salts and acidic pH, absence of hemolysis, resistant to antibiotics, able to sugar fermentation and nitrate reduction. The molecular analysis results showed that the isolate 99% similar to *Limosilactobacillus fermentum* (CECT 562(T) AJ575812) in terms of genetic affinity and the nucleotide sequence of the isolate was registered in the gene bank with the accession number (OQ568196).

### CONCLUSION

The isolated bacteria from traditional Maragheh yogurt showed probiotic potential and could be apply in food industry.

**Keywords:** Biochemical test, Intestinal flora, Microbial identification

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Immunoinformatics study on SARS-CoV-2 spike RBD for development a potent multi-epitope vaccine (MEV)

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Human coronaviruses are well adapted to humans. However, new infectious viruses could evolve, such as 2019-nCoV. This novel virus was nominated as SARS-CoV-2 by the ICTV. Its spike glycoprotein encompassing S1 and S2 subunits is the main determinant of virus neutralization. Among this, S1 receptor-binding domain (RBD) becomes as an important target for the vaccine development. So, neutralizing antibodies (Nabs) and T-cell immune responses could be induced by its multiple epitopes [1,2,3]. Accordingly, this study was aimed to predict the RBD B-Cell epitopes (BCEs) and their immunoinformatics properties for development a potent multi-epitope vaccine (MEV) in the next studies.

### MATERIALS AND METHODS

The spike Refseq (accession number: YP\_009724390.1) was aligned by the NCBI database to determine the RBD. Then, the most potent linear/conformational BCEs were predicted using a multi-method approach via IEDB, ElliPro, Discotope, ABCpred, LBtope, and CBtope servers. The antigenicity, allergenicity, toxicity, and topology of these BCEs will also be investigated to highlight their importance [4-6].

### RESULTS AND DISCUSSION

It was predicted that the spike glycoprotein contains many epitopes; however, the linear BCEs which predicted in the RBD were as follows: 2 epitopes by the Bepipred Linear Epitope Prediction, 2 highly antigenic epitopes by Kolaskar & Tongaonkar algorithm, 2 epitopes by ElliPro, and 4 epitopes showed surface accessibility by Emini Surface Accessibility. Moreover, 22, 10, and 4 potential linear BCEs were predicted by LBtope, ABCpred, and CBTOPE, respectively. On the other hand, according to the Discotope and Ellipro, the 32, and 55 potential conformational epitopes located in the RBD, respectively. As it was shown multiple epitopes were located in the RBD which are overlapped together. More studies are doing to predict their probable roles to elicit immune responses.

### CONCLUSION

The spike RBD mediates virus binding to the angiotensin-converting enzyme 2 (ACE2) receptor. Due to this critical role, it becomes a good candidate for therapeutics development. On the other hand, the in-silico approaches have gained much attention to rapid epitope prediction for the efficient vaccine design. Accordingly, many anti-SARS-CoV-2 MEV have been developed. This highlights the practical importance of immunoinformatics studies [1,5,7,8]. In this regard, this work also provides new insights into BCEs of SARS-CoV-2 which will improve and strength vaccine development after the comprehensive studies.

**Keywords:** SARS-CoV-2, B-Cell epitopes, Immunoinformatics, Vaccine design, RBD, Multi-Epitope Vaccine

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# Blow Fly Larvae Mouth Hooks Cause Infiltration of Wound Exudate Along with Larvae Secretions into the Tissues Beneath the Wound and the Bloodstream, but Fortunately Sepsis Does not Occurred at all

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

When there is little exudate in the wound, the larvae orientation is horizontal, but when there is significant exudate in the wound, the larvae orientation is vertical. In the vertical orientation larvae will close their first pair of stigma and will breath with their hind stigma. However, at frequent intervals of two to three seconds, the larvae, especially during the third instar stage, will strike the wound with their mouth hooks and will scrape the wound surface with their mouth hooks, and in the process will pierce some capillaries, which will lead to small quantities of wound exudate along with larval secretions to reach the tissues beneath the wound and the capillaries. Furthermore, when larvae are in a horizontal orientation, any movement of larvae (first piercing the wound surface with their mouth hooks and then moving further in) will create small openings and transfer wound exudate together with all larval secretions into the tissues below the wound and into the human blood circulation. Because the mouth hooks do not permit the larvae to close its mouth, the oral cavity is always open and as soon as any openings appear in the wound surface, the secretions from the larvae mouth, ammonia and wound exudate will enter the tissues beneath the wound and also human blood circulation.

Every Physician is concerned about the infiltration of wound exudate into the tissues below the wound and into the bloodstream. However, since millions of years ago the unique behavior of blowfly larvae and other facultative myiasis fly larvae have caused both wound exudate and all secretions of larvae to infiltrate into the tissues below the wound and the blood circulation. Why doesn't the unique larvae behavior cause sepsis?

Certainly, this phenomenon that exists in maggot therapy is a good key to prevent and cure blood infection in humans.

### MATERIALS AND METHODS

During the entire time using maggot therapy treatment method, we observed that as soon as the larvae reached the third stage, and the mouth hooks reached the full size, a slight bleeding was observed in the wound area, as the result of mouth hooks kick the capillary wall, causing wound exudate and all larval secretions to be entered the bloodstream, and tissues beneath the wound.

Furthermore, considering that the larvae do not have any rest during all seconds of day and night, and every movement of the larvae requires the mouth hooks to be entered the wound area, therefore the patients suffer severe pain.

We must emphasize that when the larvae are small (first and second stage), due to the small size of the mouth hooks, slight bleeding (fresh blood) is never seen in the wound.

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Considering, that the wall of capillaries is very rigid, therefore the impact of mouth hooks causes the capillaries to be punctured and the wound exudate and secretions of the larvae entered to the human blood stream and tissues beneath the wound.

Ammonia is one of the most important secretions of blowfly larvae. If more than the usual number of larvae are released to the wound in Maggot Debridement Therapy (MDT) , the elevated levels of Ammonia in the bloodstream will cause “Ammonia fever. If the maggot therapist reduces the number of maggots, within approximately an hour the patient’s fever will subside. So the larvae’s unique behavior is interconnected with the human body’s response: ammonia and other larval secretions together with wound exudate reach the tissue beneath the wound and human bloodstream. How does ammonia enter the bloodstream? Ammonia reaches the blood stream by the unique behavior of the larvae. When ammonia can enter the blood circulation through the actions of the larvae mouth hooks, surely wound exudate and all other larval secretions can also enter the blood stream.

### RESULTS AND DISCUSSION

For decades, blow fly larval secretions and excretions have been recognized to kill microbes. Much progress has been made over the past 20 years, but still we probably do not know all of innate microbial compounds produced by this fly. Still it is time that we started thinking about microbial killing beyond the value to the fly or even to the wound; we should consider the value of antimicrobial to the living host. Microbes easily and frequently enter the body through perforations in the skin, areas where the skin integrity has been disrupted, such as a skin wound. Once the microbe gains access to the blood stream(called bacteremia),it can spread throughout the entire body ,causing significant systemic complications(called sepsis)such as hypotension, cardiac failure, and death. Larval secretions not only kill microbes in the wound, but in doing, surely prevent bacteremia and sepsis. Not only do the maggot secretions and excretions dissolve the necrotic tissue where those microbes live and produce, but they also spill over the wound edges, destroying the microbes in the neighboring regions and bloodstream.

### CONCLUSION

The larval behavior will elevate the human immune system, because a small number of microbes continuously enter the tissue beneath the wound and enter the human bloodstream, the human immune system can fight efficiently against various microbes, also the larvae’s vital secretions will kill microbes in tissues below the wound and in the bloodstream.

The unique larval behavior will cause the reconnection of the wound to the human body. In fact, chronic wounds gradually cause the isolation of the wound from the rest of human body, but this beneficial process will cause all body parts, especially the human bloodstream, to be exposed to various bacteria and other pathogens that may exist in the wound.

In natural myiasis where from sunrise to sunset adult flies transfer all sorts of microbial species of nearby environment to the wound, creatures will find the ability to defend against various pathogens. Furthermore, in natural myiasis the vital secretions of adult flies will be released into the wound.

Obviously blow fly can and do cure wounds in all creatures of the world, including mammals, birds, reptiles, etc. The discussion here is centered only on human wounds and MDT. Clearly, the mechanics of the mouth hooks of blow fly larvae cause their oral secretions and ammonia in combination with wound exudate to reach the tissue beneath a wound site and blood stream of the host, be in human or animal.

**Keywords: Maggot Therapy-Sepsis-Bloodstream-Blood Infection**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Fermentative production of lysine by *Corynebacterium glutamicum*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The industrial microbiological production of the L-lysine amino acid through fermentation is of significant importance. This amino acid finds wide application in food supplements, additives, therapeutic agents, peptide synthesis precursors, agricultural chemicals, and various industries. Consequently, optimizing and economizing its production process is crucial. One of the primary sources of this amino acid is the genetically modified strain of *Corynebacterium glutamicum*. This study aimed to optimize the use of different carbon sources at varying concentrations to enhance the production of L-lysine.

#### MATERIALS AND METHODS

The study utilized *Corynebacterium glutamicum* PTCC 1606, a double auxotrophic mutant (Met<sup>-</sup>, Thr<sup>-</sup>) procured from the Iranian Research Organization for Scientific and Technology. Optimization of L-lysine production was achieved through the use of varying carbon sources at different concentrations. The Chinard method was employed for the analytical estimation of L-lysine acid, while the sugar content of the production culture was measured using the DNS method. The process was subsequently scaled up to a 10-liter fermenter in batch and fed-batch modes.

#### RESULTS AND DISCUSSION

The optimized culture medium contained 13% (w/v) dextrose. It was observed that a two-stage fed-batch fermentation using 5% dextrose and 1% CSL resulted in L-lysine production ranging between 72 to 91 g/L. The lysine production by this condition was 57g/l more than the batch culture. The findings suggest that dextrose, as a carbon source, positively impacts the production of lysine. Furthermore, *C. glutamicum* produces L-lysine, L-methionine, L-threonine, and L-isoleucine amino acids via the aspartate pathway. Using the double auxotroph strain for L-methionine and L-threonine enhances carbon flux in favor of lysine production.

#### CONCLUSION

The study data suggests a 60% increase in lysine production. Increasing dextrose and the presence of L-methionine and L-threonine in the culture medium of the auxotrophic mutant *C. glutamicum*, in conjunction with sufficient aeration in the fermenter, augments the production of L-lysine. Obviously, A two-stage fed batch culture create a significant increase in the product concentration.

**Keywords:** *Corynebacterium glutamicum*, L-lysine, fed-batch fermentation, auxotrophic mutant

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Frequency of *Pseudomonas aeruginosa*, its antibiotic susceptibility pattern and biofilm formation in fecal specimens of patients with inflammatory bowel disease and colorectal cancer compared to healthy ones

Mahtab Mehboodi<sup>1</sup>, Fereshteh Saffari<sup>2\*</sup>, Roya Ahmadrajabi<sup>3</sup>, Bijan Ahmadi<sup>4</sup>, Mohammad Javad Zahedi<sup>5</sup>

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

The intestinal carriage of *Pseudomonas aeruginosa* is likely a consequence of the opportunistic nature of this species. It is a common colonizer of the human intestine upon hospitalization and antibiotic treatment. *P. aeruginosa* may induce epithelial cell apoptosis, inflammation and cancer-related epithelial phenotypes in genetically predisposed hosts. The aim of this study was to determine *P. aeruginosa* fecal carriage rate in healthy individuals and in patients with inflammatory bowel disease (IBD) and colorectal cancer (CRC) and to detect the ability of biofilm formation and antibiotic susceptibility patterns in these isolates.

### MATERIALS AND METHODS

A total of 74 stool samples from confirmed IBD(n=48) and CRC(n=26) patients and 74 stool samples from healthy volunteers admitted to Kerman University affiliated hospitals were collected. CRC patients had not received any chemotherapy or radiation therapy and none of the participants had not antibiotic treatment in the last month. *P. aeruginosa* isolates were identified by conventional biochemical tests. Microtiter plate assay was performed for assessment of biofilm production. Disk diffusion and the microdilution broth methods were employed to determine the antibiotic susceptibility pattern of isolates.

### RESULTS AND DISCUSSION

Carriage rate with *P. aeruginosa* was 16.2%, 12.5% and 15.38% in healthy individuals, IBD and CRC patients, respectively. The ability of strong biofilm formation was seen in all isolates from CRC patients while it was 66.6% in isolates from IBD patients and healthy people. However, there were no significant differences between these groups. All isolates were susceptible to ceftazidime, cefepime and colistin and more than 90% of isolates were susceptible to piperacillin tazobactam. Antibiotic susceptibility pattern was different between isolates from two groups: the highest rates of non-susceptibility were found against levofloxacin (83.3%), piperacillin (75%), and meropenem and aztreonam (each of 66.6%) in isolates from healthy subjects while in isolates from patients, the most prevalent non-susceptibility was found against meropenem (66.6%), amikacin and gentamicin (each of 58.3%). Only one isolate from healthy group, showed multidrug resistance.

### CONCLUSION

As the titter, distribution and even virulence of *P. aeruginosa* strains may be dynamic in intestinal environment, repeatedly assessment should be designed to clarify the role of these strains in development of gastrointestinal diseases.

**Keywords:** *Pseudomonas aeruginosa*, inflammatory bowel disease, colorectal cancer, stool

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Frequency of *Peptostreptococcus stomatis* and enterotoxigenic *Bacteroides fragilis* in patients with inflammatory bowel disease and colorectal cancer compared to healthy subjects

Mahtab Mehboodi<sup>1</sup>, Roya Ahmadrajabi<sup>2\*</sup>, Fereshteh Saffari<sup>3</sup>, Bijan Ahmadi<sup>4</sup>, Mohammad Javad Zahedi<sup>5</sup>

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

The intestinal microbiota, composed of a large population of microorganisms, is often considered as “forgotten organ”. Increasing evidences indicate that dysbiosis of the intestinal microbiota is closely related to inflammatory bowel disease (IBD) and colorectal cancer (CRC). Enterotoxigenic *Bacteroides fragilis* (ETBF) may play a role in the occurrence and progression of colorectal precancerous and cancerous lesions via modulating the mucosal immune responses and inducing changes in structure and physiology of epithelial cells. Also, Although *Peptostreptococcus stomatis* (*P. stomatis*) forms part of the commensal microbiome of the human mouth and gut, it is associated with colorectal cancer through an unknown role. This study aimed to determine the frequency of ETBF and *P. stomatis* in fecal samples of patient with IBD or CRC and healthy individuals.

### MATERIALS AND METHODS

In this cross-sectional study, from August 2022 to July 2023, fecal samples were collected from healthy controls (N = 74), and patients with IBD (N = 46) and CRC (N = 28) at the time of colonoscopy admitted to Kerman University affiliated hospitals. All of the patients didn't start chemotherapy or radiation therapy for treatment. Assessment of *16SrRNA* and metaloprotease enterotoxin (*bft*) genes by PCR technique was used for detection of *P. stomatis* and ETBF isolates, respectively.

### RESULTS AND DISCUSSION

The frequency of ETBF among IBD, CRC and control cases was 28.3%, 14.3% and 17.6%, respectively ( $P > 0.05$ ). *P. stomatis* was found among 56.5%, 60.7% and 31.1% of IBD, CRC and control cases, respectively. Colonization by both ETBF and *P. stomatis* occurred in 6.7% of the healthy controls, 21.7% of the IBD and 14.3% of the CRC patients. There was a statistically significant difference in the presence of *P. stomatis* in IBD and CRC cases vs. control ones ( $P = 0.004$ ).

### CONCLUSION

The results show the association between the presence of fecal *P. stomatis* and intestinal disorders and its elevated levels may be a risk factor for developing CRC. So, detection of *P. stomatis* may provide a potential marker for CRC diagnosis. However, additional investigations on tumor and paired normal tissue samples are required to substantiate this finding.

**Keywords:** *Peptostreptococcus stomatis*, Enterotoxigenic *Bacteroides fragilis*, inflammatory bowel disease, colorectal cancer, stool

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The therapeutic effects of herbal medicines on leishmaniasis lesion

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Leishmaniasis lesion is one of the common diseases in tropical regions of the world and is caused by parasites of the genus *Leishmania*. Despite routine treatments with chemical drugs and their side effects, herbal medicines can be the best option for treatment due to beneficial properties and fewer side effects.

#### MATERIALS AND METHODS

In this study, scientific databases such as PubMed, Science Direct, Google Scholar and domestic scientific databases in Iran have been studied from 2015 to 2022.

#### RESULTS AND DISCUSSION

Based on numerous studies, herbal medicines such as *Camellia sinensis*, *Thalictrum alpinum*, *Glycyrrhiza glabra*, *Xanthium stramonium*, *Berberis vulgaris*, *Crataegus microphylla*, *Annona haematantha*, extract of *walnut*, concocted herbal (aloe vera, *perovskia abrotanoides*, propolis and lavender) have been more anti-leishmanial properties which have fewer side effects with more cost-effective and the potential to be combined with chemical substances against leishmania.

#### CONCLUSION

Herbal medicines can be one of the best options for treating leishmaniasis because these plants have less negative effects on the patient. A more effective treatment in further studies can be achieved in combination with chemical drugs.

**Keywords:** Leishmaniasis lesion, Herbal medicine, Treatment

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Screening of biosurfactant producing bacteria from diesel-contaminated soils in Kerman, Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Biosurfactants are microbial compounds that exhibit emulsifying activities. These amphipathic molecules have variety applications in biotechnology such as biodegradation and detoxification of industrial effluents, bioremediation and enhanced oil recovery due to their emulsification, cleansing, surface activity. Biosurfactants have also shown their antimicrobial and antibiofilm properties in red Biotechnology. Accordingly, the aim of this research was to isolate biosurfactant producing bacteria and their products from diesel-contaminated soils.

#### MATERIALS AND METHODS

Ten grams of diesel contaminated soil was cultivated in 90 ml of Bushnell-Haas broth containing 2% diesel fuel as the carbon source and aerated at 150 rpm in shaker incubator for 3 days adjusted on 30°C. Serial diluted grown cultures were inoculated on nutrient agar plates by spreading method and incubated at 30°C during 24 hours to achieve pure cultures. Primary tests such as hemolytic activity, emulsification, drop collapse assay, oil spreading and biochemical activities were evaluated about each isolate. Three selected isolates were cultivated in broth at 30°C and 150 rpm. After 10 days, grown cultures were centrifuged for 15 min at 8000 rpm. Cell-free supernatant was adjusted to pH: 2 with 6M HCl and stored overnight at 4°C. Precipitated biosurfactants recovered using methanol and chloroform (1:2 v/v). Hydrophobic phase was separated using funnel and dried at 50°C to obtain dry mass.

#### RESULTS AND DISCUSSION

Fifteen Gram-negative and three Gram-positive bacteria were isolated. They were monitored without hemolytic activity, beta and alpha hemolysis as 63.15%, 21.07% and 15.78%, respectively. Oil Spreading revealed a range between 3-12 mm. Out of 19 isolates 3 ones showed best results related to the primary tests. The best emulsifier isolate as IAUK101 was able to produce 9.15 grams of dry biosurfactant from 500 ml broth medium.

#### CONCLUSION

Three selected isolates had emulsion more than 50% and showed high efficiencies in biosurfactant production. These biosurfactants have a good potential to be use in bioremediation of hydrocarbons contaminated environments and further related biotechnological purposes.

**Keywords:** Biosurfactant, Diesel-contaminated soils, Emulsification, bioremediation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The effect of *Lactobacillus brevis* supernatant on the growth rate of C139 colorectal cancer cell line after 24 hours

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Lactobacilli are the normal microbiota of the digestive system. Approximately 56 species of *Lactobacillus* genus have been identified. Lactic acid bacteria can produce antimicrobial compounds and are effective against a wide range of pathogens. In addition, the anticancer effects of probiotics have been reported. They have a crucial role in preventing the transformation of pro-carcinogens into carcinogens, such as binding and reducing the absorption and inactivation of mitogenic compounds, reducing the growth of pro-carcinogenic bacteria, and increasing the function of the immune system.

This study aimed to determine the effect of *Lactobacillus brevis* supernatant on the survival of the C139 colorectal cancer cell line after 24 hours.

### MATERIALS AND METHODS

*Lactobacillus brevis* IBRC-M 10818 strain was cultured in MRS broth for 24 hours at 30°C in an anaerobic jar containing CO<sub>2</sub>. The 24-hour light absorption rate of bacteria in the MRS broth culture medium was equal to 3.3. This amount equals approximately  $5.3-9.4 \times 10^9$  CFU/mL. By centrifuging the medium, the culture supernatant was obtained and lyophilized.

Also, the C139 cancer cell line was prepared, and after culturing them,  $4 \times 10^4$  cells were transferred to 96-well plates to be affected by (0.5, 0.25, 0.06, 0.03, and 0.015 mg/mL) concentrations of bacterial supernatant. After 24 hours, the results were evaluated using the MTT Assay.

### RESULTS AND DISCUSSION

Present results showed that in 24 hours, the lowest cell viability was 48% at the concentration of 0.5 mg/mL of supernatant. Also, the highest cell viability was about 119% at the concentration of 0/015mg/mL of supernatant, and during 24h, the IC50 value was calculated at 0.55 mg/mL.

### CONCLUSION

An increase in the concentration of *Lactobacillus brevis* supernatant is related to a decrease in the viability of C139 cells. It seems likely that *Lactobacillus brevis* supernatant can prevent the growth and increase of cells in Colorectal cancer.

**Keywords:** *Lactobacillus brevis*, supernatant, C139 cell line, MTT assay

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluation of the inflammatory response in BALB/c mice infected with attenuated strain of *Leishmania major*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Cutaneous leishmaniasis is a disease caused by a *Leishmania major*. Despite the many of vaccine design researchers, an effective vaccine based on an attenuated strain of the parasite has not been introduced. The aim of this study is to investigate the inflammatory responses against the attenuated strain of passage 4 and 7 of *Leishmania major* parasite in BALB/c mice.

#### MATERIALS AND METHODS

In this study, passages 4 and 7 of the standard strain of *Leishmania major* parasite were injected into the base of the tail of female BALB/c mice, after the appearance of a skin lesion, tissues from wound, blood, spleen, liver and lymph node were evaluated on days (1, 15, 30, 45) for the expression of IL-12p35, IL-12p40, TNF- $\alpha$ , IL-1 $\alpha$ , IL-4, IL-17, IL-10, TGF- $\beta$ , Interferon- $\gamma$  and AIRE inflammatory genes by RT-PCR method.

#### RESULTS AND DISCUSSION

TNF- $\alpha$ , TGF- $\beta$ , IL-10 genes were expressed in mice infected with passage 4, which is similar to the expression pattern caused by infected with passage 1 (control), in mice infected with passage 7, the expression of TNF- $\alpha$  genes but not IL-17, IL-10 and TGF- $\beta$  was observed and lesion appeared later in this passage.

#### CONCLUSION

The expression of the IL-10 gene in mice infected with the passage 7 of parasite, indicated that the attenuated strain can cause a change in the immune inflammatory response against the parasite to controlling the disease and provide the basis for investigating the using of the attenuated strain to vaccine design.

**Keywords:** Attenuated *Leishmania major*, Inflammatory response, BALB/C mice

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation and characterization of bacteriophage against “*Escherichia coli*” causing urinary tract infection

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Urinary tract infection (UTI) is one of the most common bacterial infections. The investigations estimated that 80% of UTI are caused by “*Escherichia coli*” bacteria. The global decrease in the effectiveness of antibiotics in the treatment of bacterial infections has created a new interest in the use of bacteriophages in the treatment of infections. The purpose of this research was to isolate and identify bacteriophage effective on *E. coli* strain isolated from UTI.

### MATERIALS AND METHODS

*E. coli* strains isolated from urine specimens of patients with UTI, their antibiotic susceptibility was obtained from the clinical microbiology laboratory at the Mofid hospital. They were confirmed as UPEC with the help of biochemical and PCR methods. In this study, the phage was isolated from Mofid hospital sewage, and spot test were used for validation of phage presence. Then the phage was purified and enriched using the double layer agar techniques. To determine the morphology of the isolated phage the Transmission Electron Microscopy (TEM) was used. The effect of environmental factors on the phage such as thermal stability, pH effect and stability in the presence of chloroform and different concentrations of NaCl was determined.

### RESULTS AND DISCUSSION

Isolating bacteriophage had the ability to create suitable plaques and lyse bacteria. Based on the TEM results, the isolated phage had a large polyhedral head and a short retractable tail, belonging to the Myoviridae family. The isolated phage had the best activity at 37°C. The phage has a suitable lytic activity at pH 7, inactivation phage was observed in 70°C and PH 14. Phage lytic activity increased with increasing NaCl concentration (5%-10%-15%). The phage was resistant to chloroform.

### CONCLUSION

The isolated bacteriophage had a specific lytic activity against *E. coli* caused UTI. This phage can be used in phage therapy as an alternative treatment for people with UTIs which are resistant to antibiotics.

**Keywords:** Bacteriophage, *Escherichia coli*, Phage therapy, Urinary Tract Infection

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the physico-chemical and microbial characteristics of compost obtained from anaerobic digestion of municipal solid waste

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Over the past decades, the generation of large amounts of municipal solid waste (MSW) due to extensive urbanization has become a serious challenge, especially for large cities in developing countries. The anaerobic digestion (AD) of organic fraction municipal solid waste (OFMSW) is the most promising environmentally friendly method for solving the problem of municipal solid waste. The goal of this study was to investigate the physico-chemical and microbial (fecal coliforms and *Salmonella*) properties present in a compost of municipal solid waste of Isfahan city via mesophilic AD in a pilot scale.

### MATERIALS AND METHODS

The investigated parameters included the determination pH, EC, moisture, ammonium, faecal coliform and *Salmonella*. The determination of fecal coliform and *Salmonella* in compost was done by the most probable number (MPN) method according to the national standard of Iran No. 13321-3 and 13321-2, respectively.

### RESULTS AND DISCUSSION

The pH value varied between 7 and 8, EC, moisture and  $\text{NH}_4^+$  of content compost were 3.8 ds/m, 79% and 3000-5000 mg/L, respectively. The amount of fecal coliform and *Salmonella* in OFMSW entering the anaerobic digester was  $5.02 \times 10^7$  MPN/g and 16.13 MPN/4g, respectively. After anaerobic digestion, the amount of fecal coliform and *Salmonella* in the output of the digester decreased to  $2.3 \times 10^5$  MPN/g and 1.42 MPN/4g. The results of this study show that anaerobic digestion of waste has positive effective in reducing pathogens.

### CONCLUSION

The analyses carried on the composted municipal solid waste by anaerobic digestion showed this compost has usage potential in agriculture. Due to the short time to convert municipal solid waste into compost, this method is a suitable and promising method for urban solid waste management.

**Keywords:** Anaerobic digestion, MSW, Compost, Coliforms, *Salmonella*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Biomarkers as rapid screening tests for the detection of sepsis

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

The infection in blood stream, known as sepsis, is one of the most critical infections which needs immediate detection and subsequent medical treatments. The routine microbiological tests are time consuming. So, using a set of biomolecules named as “panel of biomarkers” is an alternative and rapid way in order to diagnosis of infection. In this review, we aimed to describe some important biomarkers for the screening of sepsis.

##### MATERIALS AND METHODS

Available international databases, Google scholar, Pubmed and Springer link were explored. The given keywords by Medical Subject Headings (MeSH) such as “Biomarkers” and “Sepsis” were also used in searching papers. Finally, the results have been limited to last 10 years, from 2013 to 2023, and related articles were analyzed.

##### RESULTS AND DISCUSSION

The findings exhibit that the biomarkers of sepsis are mainly classified in two categories of general and specific biomarkers. General biomarkers are Erythrocyte Sedimentation Rate (ESR), white blood cells count and Ferritin which all have low-sensitivity in diagnosis of sepsis. The higher sensitivity and specificity are associated to specific biomarkers which categorized in five groups: Acute-phase proteins (Procalcitonin (PCT), C-reactive protein (CRP), lipopolysaccharide-binding protein (LBP), etc.), cytokines (IL-1, IL-6, IL-8 and TNF- $\alpha$ ), coagulation biomarkers (antithrombin, proteins C and S, D-dimers, etc.), complement proteins (C3b and C5a) and lastly, soluble receptors (sTREM-1 and suPAR). Among all of these biomarkers, procalcitonin was the most important and useful biomarker, and it has its effectiveness in cooperation with some other selective biomolecules such as CRP and sTREM-1.

##### CONCLUSION

None of the mentioned biomarkers is definitive and applicable alone. Thus, a panel of biomolecules is necessary for early diagnosis of sepsis and subsequently, starting the first line wide-spectrum antibiotics to prevent the expansion of infection until the exact identification and antibiogram testing results are obtained.

**Keywords:** Sepsis, Biomarkers, Acute-phase proteins, Cytokines, Blood coagulation factors, Complement system proteins

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Tracking, sequencing and structural investigation of the genes encoding toxins of the lacticin family (*Lactococcus lactis*) in bacterial samples isolated from whey and investigating the effect of the toxin produced on the MCF7 cell line.

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

One of the biggest health challenges of human societies is the resistance of prokaryotic and eukaryotic cells to drugs. The use of probiotics and bacterial toxins, which can meet therapeutic goals with high specificity and very low side effects, is progressing day by day. Therefore, the aim of this study is to track, sequence and structurally investigate the genes encoding lacticin family toxins in bacterial samples isolated from whey and investigate the effect of the toxin produced on the MCF7 cell line.

#### MATERIALS AND METHODS

In this method, different whey samples were prepared and cultured using MRS medium at 37 degrees Celsius. The isolated bacteria were examined using biochemical tests and the isolates that were most similar to *Lactococcus lactis* were selected. Then the presence of lacticin gene was checked by PCR colony. In the next step, two isolates that had lacticin gene were selected and the effect of bacteriocin produced by them on *Staphylococcus aureus* bacteria was studied by well-plate method. Next, purified lacticin using dialysis bag was analyzed by SDS-PAGE and Bradford. The strain with the highest lacticin production was identified by sanger sequencing. Finally, the effects of lacticin obtained from this strain was studied on MCF-7 breast cancer cell line using MTT and scratching method.

#### RESULTS AND DISCUSSION

The results of this study determined that the selected bacterium containing the lacticin producing gene is *Lactococcus lactis*, which was registered in the NCBI database with the accession number OQ383929. The growth of this bacterium in acidic conditions (pH=2.5) did not show a significant difference with neutral conditions. It also had the ability to grow in bile salts, which indicates its resistance to acid and bile salts. Also, the results of SDS-PAGE determined that the produced lacticin has a weight of about 3.1 KDa. Examination with Bradford's reagent showed that this bacterium produces lacticin with a concentration of 4.2 µg/µl. In addition, the scratch test results revealed that cancer cells treated with lacticin grew slower than the control sample. Also, the results of MTT indicated that more than 80% of cancer cells were destroyed at a concentration of 7 µg/ml, and its 50% inhibitory concentration (IC50) is 5.2 µg/ml.

#### CONCLUSION

The results of this study showed that the bacteriocin produced by *Lactococcus lactis* bacteria isolated from whey has the ability to inhibit the growth and also destroy MCF-7 breast cancer cells, which shows the potential of this bacteriocin to fight and prevent breast cancer.

**Keywords: Probiotic, Lacticin, *Lactococcus lactis*, Breast cancer**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Effect of endophytic fungi isolated from *Rhabdosciadium aucheri* on *Aspergillus Flavus*

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#### ABSTRACT

Endophytes are organisms that live in living plant cells. The relationship they establish with the plant varies from symbiosis to the border of pathogenicity. Endophyte means "inside the plant". The using of this word includes a wide range of organisms such as bacteria, *fungi*, and insects inside plants. They can colonize in any organ of the host. Endophytic *fungi* are often referred to as asymptomatic *fungi* that can be in all plants. The genus *Rhabdosciadium* is grouped in the family *Apiaceae (Umbelliferae)*, order *Apiales*, order *Magnoliopsida*, suborder *Rosidae* and branch *Magnoliophyta*. This genus has 5 species in the world. The species of this genus are known as Shaleel in Iran. *Aspergillus flavus* is a pathogenic fungus that has a wide distribution in most of the world. This species is known for its symbiosis on cereals, legumes and nuts. This fungus is also an opportunistic pathogen of human and animals and causes *aspergillosis* in people with immune system deficiency. Therefore, the aim of this study is to investigate the effect of endophytic *fungi* isolated from *R. aucheri* on *A. flavus*. In order to investigate the effect of endophytic *fungi* isolated from *R. aucheri* on *A. flavus*, in this observational study, after isolating the endophytic *fungi* on the pathogenic fungus *A. flavus*, the effect and then the percentage of inhibition was measured. The experiment was conducted as a randomized complete block design with 3 replications. Data analysis was carried out with SPSS 18 software and the comparison of means was done with Duncan's multi-range test method at the probability level of 5%. Finally, it was found that cross-cultivation of *Alternaria rosae K11* against *A. flavus* with The inhibition rate of 87.5% indicates the highest inhibitory power and also the cross-culture of *Alternaria radicina 2K2* against *A. flavus* with inhibition rate of 12.1% indicates the lowest inhibitory power of this endophyte against *A. flavus*.

**Keywords:** Endophyte, Secondary metabolite, Fungi, *Rhabdosciadium aucheri*, Microorganism

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Safety and potential allergenicity assessment of modified bacterial CA protein for transfer to plant in order to enhancing carbon sequestration efficiency

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

The enzyme Carbonic anhydrase (CA) captures carbon by converting CO<sub>2</sub> to bicarbonate, which can serve as a carbon source for bacteria and plants. Various structures of this enzyme are found in organisms with different stability and efficiency. Transferring the optimized sequence of CA to bacteria and plants can improve carbon sequestration efficiency and enhance growth and metabolism. The purpose of this study is to evaluate the safety and potential allergenic effects of bacterial CA protein after codon optimization and its sequence modification in order to transfer to bacteria and plants to increase growth and metabolism efficiency.

##### MATERIALS AND METHODS

In this study, the CA gene sequence of *Caminibacter mediatlanticus* bacterium was used. The codon-optimized and modified nucleotide sequence of CA, in FASTA format, was investigated in allergen databases including "ALLERGEN ONLINE" and "ALLERMATCH". In this way, the complete sequence, 80-amino acid sequences, and 6-8 amino acid sequences were compared with allergen sequences in the allergen database. Furthermore, the protein sequence was subjected to *in silico* enzyme digestion using pepsin, trypsin, and chymotrypsin in the "PEPTIDE CUTTER" database, and peptide fragments longer than eight amino acids were also examined in the allergy database.

##### RESULTS AND DISCUSSION

The homology analysis of the CA protein with various allergy databases showed no allergenicity. Furthermore, *in silico* enzyme digestion of this protein using pepsin, trypsin, and chymotrypsin generated 39, 32, and 25 peptide fragments, respectively, with 11, 8, and 10 fragments longer than 8 amino acids. The examination of short peptide fragments was performed to investigate the possibility that peptide fragments larger than eight amino acids may act as epitope antigens. None of the resulting peptide fragments from the enzymatic digestion of the CA protein matched with the allergen peptides in the database.

##### CONCLUSION

According to the WHO and FAO regulations, similarity of more than 55% and 95% for the full-length and 80-amino acid sequences, respectively, with allergen sequences can be considered significant. Our results demonstrated the non-allergenic nature of this protein. Therefore, this study can address concerns related to the use of the modified sequence of this protein for transfer to different plant species.

**Keywords:** Allergenicity, Carbonic anhydrase, Modified protein, Safety

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Lipid production from a newly isolated yeast *Candida parapsilosis* EBL29 for biodiesel production

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## ABSTRACT

Fossil fuels are the main source of energy demand; however, climate changes have made a great concern to look for new and efficient renewable energy sources. Oleaginous yeasts can produce and store lipids at least 20% of their cell dry weight in the form of extracellular or intracellular single-cell oil (SCO). In the recent research, fifty-three yeast strains were screened and isolated from different environmental samples. Then, samples were cultured on Rose Bengal Chloramphenicol agar for microscopic observation of colonies. Thirty-four strains were able to lipogenesis on Oleaginous Yeast Enrichment Medium containing 5% glycerol and subsequently incubated on saponification wastewater to investigate dry biomass and lipid production. Among these strains, the lowest cell dry weight (CDW) was 4.5 gr/L and 1.27 gr/L lipid production. The most promising results with 13.5 gr/L cell dry weight and 6.75 gr/L lipid production, was related to *Candida parapsilosis* EBL29 that was identified by ITS sequencing with 99.47% homology to *Candida parapsilosis* ATCC 22019. With its 50% lipid yield (lipid production to CDW ratio), *Candida parapsilosis* EBL29 can be a potential candidate to produce the third generation of biofuels using inexpensive industrial wastewater as a growth medium.

**Keywords:** Biodiesel, Biofuel, Lipid extraction, Lipogenesis, Oleaginous yeasts, Renewable energy

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# A meta-analysis study of the occurrence of adhesion and biofilm-related genes in *Staphylococcus aureus* isolates

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### ABSTRACT

*Staphylococcus aureus*, a highly adaptable pathogen, has the capacity to cause a broad spectrum of infections, ranging from minor skin issues to severe and life-threatening invasive diseases. The pathogenic nature of *S. aureus* can be attributed to its production of various virulence factors, including proteins associated with adhesion and biofilm formation.

In this particular study, we conducted an extensive network meta-analysis to investigate the prevalence of adhesion and biofilm-related genes within *S. aureus* isolates. Additionally, we explored how the source of the isolate affected the occurrence of these genes.

A total of 53 relevant studies were included in our analysis. Among the genes studied, *clfB* showed the highest prevalence (p-estimate = 85.4, CI95% 78-90.6), followed by *eno* (p-estimate = 81.1, CI95% 61.7-91.9), and *icaD* (p-estimate = 77, CI95% 68.6-83.6). On the other hand, *bap* and *bbp* genes displayed the lowest prevalence rates (p-estimate = 6.7 and 18.7, respectively). We also observed that the gene pairs *icaA-icaD* (30 times) and *fnbA-fnbB* (25 times) were the most frequently co-studied.

Intriguingly, the subgroup analysis demonstrated that the occurrence of *icaC* and *icaB* genes was significantly lower in animal isolates when compared to human and food isolates ( $p < 0.05$ ). However, it is essential to note that there was a limited amount of data available for the analysis of *sasG*, *bbp*, *bap*, *eno*, and *fib* genes.

Through our study, we have uncovered varying prevalence rates of adhesion and biofilm-related genes in *S. aureus* isolates. These findings contribute significantly to our understanding of *S. aureus* pathogenesis and can play a crucial role in the development of effective prevention and treatment strategies for *S. aureus* infections.

**Keywords:** *Staphylococcus aureus*; Adhesion genes, Biofilm formation, Gene prevalence, Comparative analysis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of *Rickettsia* species in the WHO Regional Office for the Eastern Mediterranean (EMRO): an overview

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Rickettsia* is a zoonotic bacterial pathogen which transmitted by vectors and has extensive reservoirs in animal and human populations. Rickettsiosis is a public health problem all over the world. However, comprehensive information on the geographical distribution of different *Rickettsia* species, contamination status of reservoirs, vectors, and human cases is lacking in most parts of the world. Therefore, the aim of this study was to investigate the geographical distribution of different *Rickettsia* species and their vectors in countries of the WHO-EMRO region.

### MATERIALS AND METHODS

In this review study, a search was conducted for reports and published studies on *Rickettsia* species from WHO-EMRO region countries in various databases from 1995 to 2022. Finally, the reported status of human cases, reservoirs, and vectors associated with each species in different countries was documented.

### RESULTS AND DISCUSSION

Reports of contamination related to the detection of *Rickettsia* species were only available for 15 out of 22 WHO-EMRO member countries. In total, twenty-four different *Rickettsia* species, including *R. sibirica*, *R. lusitaniae*, *R. africae*, *R. prowazekii*, *R. felis*, *R. typhi*, *R. rickettsii*, *R. aeschlimannii*, *R. conorii*, *R. massiliae*, *R. helvetica*, *R. monacensis*, *R. rhipicephali*, *R. bellii*, *R. asembonensis*, *R. hoogstraalii*, *R. andeanae*, *R. raoultii*, *R. asiatica*, *R. slovacica*, *R. australis*, *R. barbariae*, *Candidatus R. amblyommii*, and *Candidatus R. goldwasserii*, were reported from WHO-EMRO member countries. Additionally, human cases which were infected with six different *Rickettsia* species including *R. sibirica*, *R. prowazekii*, *R. felis*, *R. typhi*, *R. rickettsii*, *R. aeschlimannii*, *R. conorii*, *R. massiliae*, and *R. helvetica* reported from these countries.

### CONCLUSION

According to the wide range of vectors and the abundance of *Rickettsia* species, the investigation of rickettsial infections in the WHO-EMRO region is important but has been neglected. Therefore, it should be taken seriously.

**Keywords:** *Rickettsia*, Rickettsiosis, WHO-EMRO, Zoonosis, Epidemiology.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation of *Lactiplantibacillus plantarum* from Markhoz goat feces and determination of probiotic activities of this bacterium

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#### ABSTRACT

Probiotics are living and non-pathogenic microorganisms that are part of the intestinal microbial flora. Probiotics are composed of different species, the most important of which are *Lactobacillus* and *Bifidobacterium*. The aim of this study is to investigate the probiotic potential of *Lactobacillus* isolated from Markhoz goat feces [1,2].

First, freshly prepared feces were collected through a sterile strainer. After centrifuging the liquid passed through the filter at 3000 rpm for 2 minutes, the liquid was inoculated in MRS broth culture medium. Then the samples were incubated for 24 hours at 35°C. Then, this medium was sub-cultured on MRS agar. Grown colonies were used to perform diagnostic microbial tests including Gram staining, catalase test and fermentation of carbohydrates. Finally, for the final confirmation of the suspected *Lactobacillus* bacteria, the species-specific PCR and subsequently gene sequencing was applied.

The obtained sequence was identified in the NCBI database as *Lactiplantibacillus plantarum*. After isolating the bacteria, its probiotic activity including bile tolerance, salt tolerance (3 and 6%), pH tolerance: 2.5 for 2 hours, antimicrobial properties against *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* were confirmed.

The present study showed that the feces of native goats of Kurdistan province (Markhoz goat) contain *Lactobacillus* bacteria that have high probiotic power and these bacteria can be used in the design a new drug, especially in the field of fighting against antibiotic resistant pathogens.

**Keywords:** *Lactiplantibacillus plantarum*, Markhoz goat, feces, pathogen, probiotic properties

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and introducing *Candida* SP. PZ9 is a resistant strain to high concentrations of zinc and its potential for practical use in food supplements.

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#### ABSTRACT

Trace elements cannot be obtained in sufficient quantities from food alone to meet the human body's nutritional demands, resulting in a constant state of inadequacy of these elements in the body. Because inorganic zinc is challenging for mammals to absorb and utilize in the gastrointestinal tract, it is preferable to use the organic form. This study aims to enrich zinc in *Candida* sp. PZ9 produces yeast rich in zinc and uses it in the food industry and animal and human feed.

A total of 50 samples of yeast were examined to determine their capacity for growth in varying concentrations of zinc. One strain that displayed a high level of resistance to high zinc concentrations was chosen after conducting screening tests. This particular strain was sent for molecular identification, which revealed it to be *Candida* SP. PZ9 is a type of yeast that can withstand zinc concentrations of up to 4000 ppm. Following the identification, the yeast was cultured and its dry weight and atomic absorption were measured. The measurements of dry weight at a concentration of 2000 ppm, 3000 ppm, and 4000 ppm of zinc, were 14.5 g L<sup>-1</sup>, 8 g L<sup>-1</sup>, and 4.5 g L<sup>-1</sup>. In addition, according to the AAS results, the sample contained 1900 ppm of zinc, indicating that the yeast cells had absorbed this amount of the zinc metal, which shows the high ability of metal absorption by this yeast. Moreover *Candida* SP. PZ9 grows well in medium containing concentrations of 1000 ppm and 2000 ppm of other metals such as copper, selenium, and chromium.

Due to the mentioned properties of this yeast in absorbing zinc, high biocompatibility, and easy absorption, upon obtaining the necessary approvals, it has the potential to be used as a nutritional supplement for animal and human feed.

**Keywords: Biosorption, Bioaccumulation, Enrichment yeast, Food supplements**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The effect of breast milk on the oral microbiome: short-term and long-term effects

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The human oral microbiome plays an essential role in human health. Oral bacteria during infancy can affect bacterial colonization in the future. Environmental and nutritional factors, such as breast-feeding have a significant impact on the development of the infant's oral microbiome. Breast milk is not sterile, but it contains a source of specific microbiota for the baby. *Streptococcus* spp. and *Lactobacillus* spp. are common bacterial species found in breast milk. There are few studies on whether the effect of breastfeeding on the infant's oral microbiota is long-term or short-term. The goal of the present review is to investigate the effect of breastfeeding on oral microbiota in infants and adults.

### MATERIALS AND METHODS

In this study, the effect of breast milk on human oral microbiota has been investigated, by reviewing the other literature.

### RESULTS AND DISCUSSION

The pattern of oral microbiota is different between breast-fed and formula-fed infants. *Streptococcus mutans* is more common in formula-fed infants. Some microbial species will remain even after the cessation of breast-feeding, showing the long-term effect of breastfeeding on the oral microbiota. It is known that the mother's oral health is effective on the transfer of bacteria from mother to baby. Although the mother's microbiome is one of the main microbial sources at the beginning of the baby's life, the transmission of the microbiome between the mother and the baby is not well studied yet.

### CONCLUSION

Bacteria in breast milk, especially probiotics, play an essential role in the oral health of infants; however, more studies are needed to understand its long-term effects.

**Keywords: Breast milk, Oral microbiota, Long-term effects**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Synthesis of CDs by hydrothermal method and evaluation of its anti-bacterial and anti-biofilm effect against antibiotic-resistant *S. aureus* and *P. aeruginosa* strains

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Antimicrobial resistance and biofilm formation are becoming one of the most important public health. Therefore, the prevention of their formation has been a serious challenge. The present study focuses on understanding the new strategy to inhibit biofilm formation and explores the potential role of *Echium italicum* -derived carbon dots (CDs) as biofilm inhibitor compound.

### MATERIALS AND METHODS

The minimal inhibitory concentration (MIC) of CDs was determined by broth microdilution method. The antibacterial activity of CDs in combination with clindamycin and ciprofloxacin was evaluated using the checkerboard method. By microtitre plate method, the anti-biofilm effect of CDs alone and in combination with clindamycin and ciprofloxacin was also evaluated. Then the toxicity of each of the agents was investigated on L929 fibroblast cells. Finally, the effects of CDs on the expression of *pslA*, *pelA* and *ppyR* genes in *P. aeruginosa* and *icaA*, *icaC*, *icaD* and *bap* genes in *S. aureus*, which are related to biofilm formation were determined using RT-q PCR.

### RESULTS AND DISCUSSION

The synthesized CDs had an emission maximum of 455 nm and the average diameter of the CDs was estimated to be  $3.4 \pm 0.5$  nm. CDs showed the lowest inhibitory concentration (MIC) between 125-500  $\mu\text{g/ml}$  against clindamycin-resistant *S. aureus* isolates and between 0.5-1 mg/ml against ciprofloxacin-resistant *P. aeruginosa* isolates. CDs at MIC and sub-MIC concentrations significantly prevented biofilm formation in *P. aeruginosa* ( $P < 0.0477$ ,  $P < 0.0280$ ) and *S. aureus* ( $P < 0.0082$ ,  $P < 0.0055$ ). Also, the combination of CDs with ciprofloxacin and clindamycin significantly reduced growth ( $P < 0.0001$ ) and inhibited biofilm formation in clindamycin-resistant *S. aureus* and ciprofloxacin-resistant *P. aeruginosa* isolates ( $P < 0.0053$ ,  $P < 0.01$ ). In addition, the ability of the antibiotics clindamycin and ciprofloxacin decreased by approximately 98% within 72 hours. The toxicity of the antibiotics also decreased in combination with CDs. The expression of *pslA*, *pelA* and *ppyR* genes in *P. aeruginosa* and *icaA*, *icaC*, *icaD* and *bap* genes in *S. aureus* decreased significantly after exposure to CDs ( $P < 0.0023$ ,  $P < 0.0045$ ).

### CONCLUSION

This study revealed a promising new method for the treatment of chronic infections. In addition, by minimizing the dose of antibiotics, the toxic effects of dose-dependent antibiotics and the antimicrobial activity can be improved.

**Keywords:** Resistant. *P. aeruginosa*, *S. aureus*, Carbon dots, *Echium italicum*, Biofilm

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Optimization of carotenoid production in native *Rhodococcus* strain using different carbon sources and amino acids

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Microbial pigments have been applied in various industries such as food and etc, and these compounds show antimicrobial, anticancer activities because they are less toxic than their synthetic analogs. The actinomycetes genus *Rhodococcus* is one of the most important pigment-producing bacteria. The metabolic diversity of *Rhodococcus* spp. strains has been expanded for their biosynthetic capabilities, which can be used in biofuel generation, metal recovery, and novel drug discovery. This study aims to optimize the production of carotenoid pigment in native strains of *Rhodococcus* isolated from soil around a manganese mine in Qom City by Aghaei *et al.*

### MATERIALS AND METHODS

Study of pigment production in culture media such as TSA, BHI Agar, LB Agar, Bennet Agar, and ISP5 at different temperatures of 25 °C, 30 °C, and 35 °C; amino acids including aspartic acid, glutamic acid, tryptophan, and tyrosine with concentrations of 0.5%, 1%, and 1.5%; different carbon sources including glucose, sucrose, maltose, and lactose with concentrations of 0/125%, 0/25 %, 0/5%, 1%; and different nitrogen sources consisting of sodium nitrate, ammonium nitrate, beef extract with concentrations of 0/5%, 1%, 1/5%; and other factors such as pH (5, 6, 7, 8) and the rpm of the incubator shakers at 150, 180, and 200 in the native strain of *Rhodococcus rhodococcus*.

### RESULTS AND DISCUSSION

In this research, the medium TSA has been identified as the best medium in terms of the growth of the strain *Rhodococcus* and the production of its pigment. The statistical analysis showed that the best optimal conditions for the production of carotenoid pigment are: a temperature of 30 °C, an rpm of 200, pH = 7, maltose 1%, sodium nitrate 1/5%, and tryptophan 1/5%.

### CONCLUSION

According to the results of this research, Native *Rhodococcus rhodochrous* isolated can be considered as a good candidate for carotenoid pigment production with biotechnological application in food and pharmaceutical industries.

**Keywords:** Optimization-Carotenoid pigment- Native *Rhodococcus*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibiotic resistance, biofilm formation, and biofilm-associated genes among *Stenotrophomonas maltophilia* clinical isolates

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Stenotrophomonas maltophilia*, previously known as *Pseudomonas maltophilia*, has become nowadays a major opportunistic pathogen in hospitalized or immunocompromised patients worldwide. The present study was to investigate the antimicrobial susceptibility pattern, biofilm production, and the presence of biofilm genes among the *S. maltophilia* clinical isolates.

### MATERIALS AND METHODS

A total of 85 clinical isolates of *S. maltophilia* were collected from patients referred to several hospitals. Susceptibility to antibiotics was investigated by disc diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). By the crystal violet staining method, the capability of biofilm formation was examined. The genes associated with biofilm production were investigated by the PCR-sequencing techniques.

### RESULTS AND DISCUSSION

All isolates were resistant to doripenem, imipenem, and meropenem. Minocycline, trimethoprim/sulfamethoxazole and levofloxacin exhibited the highest susceptibility of 100%, 97.65%, and 95.29%, respectively. The results of crystal violet staining assay showed that all isolates (100%) form biofilm. Moreover, 24 (28.23%), 32 (37.65%), and 29 (34.12%) of isolates were categorized as weak, moderate, and strong biofilm producers, respectively. Biofilm genes including *rpfF*, *spgM* and *rmlA* had an overall prevalence of 89.41% (76/85), 100% (85/85) and 84.71% (72/85), respectively.

### CONCLUSION

Rational prescribing of antibiotics and implementation of infection control protocols are necessary to prevent further infection and development of antimicrobial resistance. Combination strategies based on the appropriate antibiotics along with anti-biofilm agents can also be selected to eliminate biofilm-associated infections.

**Keywords:** *Stenotrophomonas maltophilia*, Antibiotic resistance, Biofilm, Biofilm formation genes

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The controversial association of gut microbiota with kidney stone formation

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#### ABSTRACT

Nephrolithiasis (kidney stones) is one of the most common chronic kidney diseases that are typically more common among adult men comparing to adult women. The prevalence of this disease is increasing which is influenced by genetic and environmental factors. Kidney stones are mainly composed of calcium oxalate and urinary oxalate which is considered a dangerous factor in their formation. Besides diverse leading reasons in the progression of nephrolithiasis, the gut microbiome has been recognized as a major player in the development or prevention of it. These microbes produce metabolites that have diverse effects on host biological functions. Therefore, Changes in the composition and structure of the microbiome (dysbiosis) have been implicated in various diseases. The present review focuses on the roles of gut microbiota in kidney stone formation.

**Keywords:** Nephrolithiasis, gut microbiota, kidney stone, microbiome

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Characterization of isolated probiotic bacteria from traditional Maragheh yogurt

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

According to the definitions of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), probiotics as living microorganisms by maintaining or improving the microbial balance, can confer health benefits on their host. Probiotic bacteria regulate the gut microbiota by adhering to the intestinal epithelial tissues, enhancing nutrient uptake, synthesizing vitamins and short-chain fatty acids, and modulating the pH of the gut.

### MATERIALS AND METHODS

After isolation and purification, probiotic properties of the M20 isolate were evaluated by gram staining, oxidase, catalase, hemolysis, and acid and bile salt resistance tests. Also, microbial tests including nitrate reduction, SIM, sugar fermentation, antibiotic resistance and antimicrobial were conducted. For molecular identification of the isolate, DNA was extracted using a kit, PCR amplification of 16S rRNA gene performed, electrophoresed and phylogenetic tree of the isolate constructed by neighbor-joining method by BioEdit and MEGA version 7 softwares.

### RESULTS AND DISCUSSION

According to the results, the M20 isolate was known as a probiotic bacteria due to gram-positive morphology, negative results for catalase and oxidase tests, resistance to bile salts and acidic pH, and absence of hemolysis. The microbial tests indicated that the isolate was resistant to antibiotics and capable of sugar fermentation and nitrate reduction. Based on the molecular identification result, the isolate showed highest genetic similarity (86%) to *Lactobacillus plantarum* DMS 10667 (T) CP032744. The nucleotide sequence of the M20 isolate has been deposited in the NCBI database under accession number OQ568197. Moreover, the production of asparaginase and glutaminase by the M20 isolate, which are known therapeutic enzymes, was confirmed.

### CONCLUSION

The lactic acid bacteria isolated from traditional Maragheh yogurt exhibited probiotic properties, could be considered as a favorable candidate to use in food and pharmaceutical industries.

**Keywords:** Dairy product, *Lactobacillus*, Probiotic

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Gram-negative Bacteria's Small Non-coding RNAs: New Insights and Comprehensive Review of Mechanisms, Functions, and Potential Applications

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## ABSTRACT

Small non-coding RNAs (sRNAs) are a key part of gene expression regulation in bacteria. Many physiologic activities like adaptation to environmental stresses, antibiotic resistance, quorum sensing, and modulation of the host immune response are regulated directly or indirectly by sRNAs in gram-negative bacteria. Therefore, sRNAs can be considered as potentially useful therapeutic options. They have opened promising perspectives in the field of diagnosis of pathogens and treatment of infections caused by antibiotic-resistant organisms. Identification of sRNAs can be executed by sequence and expression-based methods. Moreover, computationally prediction of sRNAs using bioinformatic tools is developed in recent years, which helps to get more comprehensive data in combination with experimental approaches.

Despite the valuable progresses in the last two decades, and discovery of new sRNAs, their exact role in biological pathways especially in corporation with other biomolecules involved in gene expression regulation such as RNA-binding proteins (RBPs), Riboswitches, and other sRNAs needs further investigation.

Although there are many RNA databases that RNAcentral uses 59 of them, the lack of a professional database to categorize current experimentally validated sRNAs in gram-negative pathogens is a gap. Here we review the present knowledge on most recent and important sRNAs and their regulatory mechanism, strengths and weaknesses of current methods of sRNAs identification, and at the end, demonstrate potential applications and new insights of sRNAs for future studies.

**Keywords:** *Helicobacter pylori*, Gastritis, Genotyping, *vacA*, *cagA*, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# *M. tuberculosis* and SARS-CoV-2 co-infections: A review

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Tuberculosis (TB) is still one of the most important causes of death worldwide. The lack of timely attention on TB diagnosis and treatment during the Coronavirus Disease 2019 (COVID-19) pandemic is a potential threat to health issues and may have severe consequences for patients and health systems. There is not much information on the management of TB during this period. Here, we reviewed the current literature to evaluate the rate of *Mycobacterium tuberculosis* and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) co-infections and interactions between these infectious agents.

### MATERIALS AND METHODS

Several databases, including Web of Science, Scopus, and Medline (via PubMed), were searched for original articles addressing TB and COVID-19 diseases published from December 2019 to April 2021.

### RESULTS AND DISCUSSION

Out of 3879 articles, Fifty-seven articles were included in this study, and among 106,033 patients affected by COVID-19, 891 also had TB. Overall, investigators found a consistent increase in *C-reactive protein*, D-dimer (especially in patients with severe clinical manifestation), *erythrocyte sedimentation rate*, lactate dehydrogenase, alanine aminotransferase, and a reduction of lymphocytes. The respiratory symptoms of TB/COVID-19 patients were similar to those of TB, but the risk of developing pulmonary TB increased in COVID-19 patients. Also, the mortality rate in TB/COVID-19 patients was higher than patients affected only by COVID-19 or TB.

### CONCLUSION

Some reports indicated worsening respiratory symptoms and even activation of latent TB after COVID-19 or vice versa. It seems that both active and previously treated TB constituted a risk factor for COVID-19 in terms of severity and mortality, regardless of other underlying diseases and patient status. Health systems should not neglect TB during this era of the ongoing COVID-19 pandemic by setting up appropriate diagnostic and clinical management algorithms.

**Keywords:** *Mycobacterium tuberculosis*, TB, COVID-19, SARS-CoV2, Coronavirus, Co-infection

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Qualitative detection methods for identification of Polyhydroxyalkanoates (PHAs) in microorganisms

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Polyhydroxyalkanoates (PHAs) are elastomeric polyesters that accumulate as a carbon/energy storage materials in various microorganisms. Unlike petrochemical-based plastics that take several decades to fully degrade, PHAs can be completely degraded within a year by a variety of microorganisms into CO<sub>2</sub> and water. Recently much efforts have been devoted to develop a process for economical PHAs production. The isolation, analysis and characterization of PHAs are significant factors for any process development. This paper compiles the methods available for qualitative identification of PHAs.

#### MATERIALS AND METHODS

Selected strains were cultivated on MSM (Mineral Salt Media) agar and PDA (PHA-Detection-Agar). Primary screening by SBB (Sudan Black B) plate assay and SBB staining, were performed to identify the PHA fabricating potential of these isolates. For the rapid detection of PHA producing bacteria, an alcoholic SBB solution was poured above the grown colonies on the plates. Subsequently, for microscopic studies, smears of colonies were fixed on glass slides, followed by staining with SBB solution. These isolates were further subjected to secondary screening using the NBA (Nile Blue A) plate assay method and NBA staining. In the viable colony method, NBA solution as an indicator was incorporated into PDA and MSM agar. Furthermore, in another method colonies on an agar plate were stained with NBA solution. Finally, the bacterial strains by NBA fluorescence staining were examined under a fluorescence microscope.

#### RESULTS AND DISCUSSION

In Viable colony staining technique using SBB plate assay, the colonies able to produce lipid appeared bluish black. These isolates demonstrated fluorescence colonies under UV after being cultured on NBA plates. In addition, the dark blue-coloured colonies after staining with NBA solution were positive for PHAs production. Subsequently, light microscopy indicated that the PHAs granules accumulated by the isolates appeared as blue-black droplets. Fluorescent microscopy with NBA showed that PHAs inclusion bodies produce golden yellow fluorescence.

#### CONCLUSION

Research on PHAs has been encouraged by their potential use as biodegradable alternatives to petrochemical plastics. The development of new, simple and rapid methods for qualitative detection and quantification of PHAs will certainly facilitate efficient economic production processes.

**Keywords:** Polyhydroxyalkanoates, PHA-detection, Sudan black B plate assay, Nile blue A plate assay.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Interaction of Gut Microbiota and Their Metabolites in patients with sepsis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Sepsis is a complex clinical disorder with heterogeneous etiological factors. Given its high mortality rate, it is considered a global health issue. Recently, the link between gut microbiota and their metabolites, especially short-chain fatty acids, in the pathophysiology of sepsis has been reported. However, there are few findings to confirm this relationship. This study aimed to evaluate some key gut microbiota members, pathogenic bacteria, and short-chain fatty acids in non-ICU patients with sepsis caused by bacteremia compared to a control group.

### MATERIALS AND METHODS

In this case-control study, 45 stool samples from patients with sepsis and 15 healthy persons were collected from October 2021 to August 2022 in Tabriz, Iran. The position of some gut microbiota members and the main short-chain fatty acids concentration were assessed in the two groups by the Q-PCR and the high-performance liquid chromatography system.

### RESULTS AND DISCUSSION

*Faecalibacterium prausnitzii* and *Bifidobacterium* spp. As bacterial with protective features in non-ICU patients with sepsis decreased significantly. Moreover, the concentrations of acetic acid and propionic acid significantly decreased in this group compared to the healthy volunteers. In contrast, the pathogenic bacteria members such as Enterobacteriaceae and *Bacteroides* spp. Increased significantly in the patients compared to the healthy individuals. The concentration of butyric acid decreased in the patients, but this change was not significant in the two groups.

### CONCLUSION

Protective and immune functions of *F. prausnitzii* and *Bifidobacterium* spp., as well as acetate and propionate, are evident. In this investigation, this profile was significantly reduced in non-ICU patients with sepsis compared to the control group.

**Keywords:** Gut microbiota, Sepsis Short-chain fatty acids, *Faecalibacterium prausnitzii*, *Bifidobacterium* spp.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Prevalence of antibiotic resistance genes in *Staphylococcus aureus* isolated from dairy products in Shahrekord, Iran.

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Staphylococcus aureus* (*S. aureus*) is a major foodborne pathogen throughout the world and one of the most causes of food poisoning in milk and dairy products. The production of a biofilm enhances its virulence as it offers resistance to substances such as antibiotics that can inhibit its growth.

#### MATERIALS AND METHODS

A total of 90 samples of raw cow's milk were collected. Presumptive *S. aureus* strains were obtained using Baird-Parker plates after enrichment in tryptone soy broth and final colonies were selected from brain heart infusion. Additional test such as coagulase were done and identification were confirmed by the detection of the *aroA* gene. Biofilm producing strains were screened using a spectrophotometry method applied in microplates. Crystal violet staining was used to quantify the formation of biofilm. Antibiotics susceptibility test was performed using the Kirby-Bauer disc diffusion method. PCR was used to detect several biofilm and antibiotics resistance related genes.

#### RESULTS AND DISCUSSION

The results for the presence of *S. aureus* in raw cow milk show that of the 90 milk samples, 35 samples (38.88%) were positive for *S. aureus* and all isolates were approved by PCR for the presence of the *aroA* gene. Antibiotics susceptibility test show alarming rate of resistance to beta-lactam antibiotics specially penicillin (100%), ampicillin (91.42%) and oxacillin (71.42%). This finding correlate antibiotic resistance gene detection which the gene *blaZ* was the most found (71.42%) followed by *mecA* and *aac-D* (42.85%).

#### CONCLUSION

Statistical tests show a significant correlation between biofilm production and antibiotic resistance in *S. aureus*. These results highlight the need for regular surveillance of the occurrence of *S. aureus* strains in milk and milk products in Iran.

**Keywords:** Antibiotic resistance, Biofilm, Milk, *Staphylococcus aureus*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Anti-bacterial Activity of Bio-Synthesis of Cerium Oxide Nanoparticles with Gallic acid

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Cerium oxide nanoparticles, also known as nanoceria, are particles of cerium oxide that are less than 100 nanometers in size. They have unique physical and chemical properties that make them attractive for a wide range of applications, including catalysis, energy storage, biomedical function, and drug delivery. The antibacterial properties of cerium oxide nanoparticles are believed to be due to their ability to generate reactive oxygen species (ROS). The objective of this study is to investigate and characterize the antibacterial effect of cerium oxide nanoparticles (CeO<sub>2</sub>NP) synthesized using Gallic acid.

### MATERIALS AND METHODS

The biosynthesis of CeO<sub>2</sub>NP was performed using Gallic acid by sol-gel method; This method involves the reaction of a cerium nitrate with a stabilizing agent (Gallic acid), then pH of the solution adjusted to basic to 10, and heated in 400°C for 2h to form cerium oxide nanoparticles. The synthesized nanoceria was evaluated by SEM, XDR, DLS, ZETA and FTIR tests. Antimicrobial properties of CeO<sub>2</sub>NP against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella* bacteria were measured by MIC.

### RESULTS AND DISCUSSION

The results of current study shown that average size of CeO<sub>2</sub>NPs was 100-200 nm and XRD analysis displayed the cubic fluorite structure of the synthesized nanoparticles. FT-IR reveals stretching frequencies at 550 cm<sup>-1</sup> which confirmed the Ce-O stretching bands and showing application of natural components for the production of nanoparticles. The SEM images reveal that the prepared CeO<sub>2</sub>NP are composed of spherical nanoparticles in aggregated form. Minimum bactericidal concentrations for Gram positive and -negative of 100- 250µg/mL were generated. However, *S. aureus* and *E. coli* exhibited the higher sensitivity, while other were the slightest sensitive to CeO<sub>2</sub>NPs.

### CONCLUSION

These results indicate that CeO<sub>2</sub>NPs synthesized using Gallic acid are hopeful another treatment for some bacterial infection. Also, this study will give the possible for the sustained progress of biocompatible nanoparticles with improved biological abilities derived from natural products.

**Keywords:** Cerium oxide nanoparticle, Green synthesized, Anti-bacterial, Gallic acid

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation of *Stutzerimonas balearica* strain RBB5 with the ability to biodegrade BTEX

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Groundwater contamination with benzene, toluene, ethylbenzene, and xylene (BTEX) has very risk in environment and health, and requiring the urgent development of methodologies to remove these compounds. The aim of this study was to isolate an efficient strain capable of biodegrading these compounds.

#### MATERIALS AND METHODS

Sampling was done from a depth of 50 cm in oil-contaminated areas. First, the soil sample was enriched with 1% BTEX for 14 days. Next, this suspension was cultured on solid medium and its microorganisms were purified. Then, bacteria were cultured in the presence of 1% BTEX. After that, evaluation of the biodegradation of BTEX was done by Gas chromatography-mass spectrometry. Finally, Molecular identification of the isolate was done with a 16s rRNA gene primers.

#### RESULTS AND DISCUSSION

The selected bacterium had the ability to grow at temperatures of 20 to 40 degrees in pH 5 to 9 and sodium chloride concentrations of 0 to 8%. The GC-MS results showed that this bacterium could reduce benzene by 63%, toluene by 57%, ethylbenzene by 93% and xylene by 100% in the aqueous phase. Molecular identification of the isolate showed that this strain is 98% similar to *Stutzerimonas balearica*. This strain has a great ability to remove xylene (both ortho and para) and ethylbenzene, although it has less ability to remove benzene and toluene.

#### CONCLUSION

BTEX compounds are very toxic and carcinogenic. This strain can be a proper option for bioremediation due to no produce by-products.

**Keywords:** Oil pollutant, Remediation, Molecular identification, GC-MS

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Biodegradation of BTEX by a new strain of *Bacillus haynesii*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

BTEX (benzene, toluene, ethylbenzene, and xylene) are potentially carcinogenic compound. Other derivatives of these compounds are also known to be cause toxic in nerve and respiratory systems. For this reason, the objective of this study was to assess the biodegradation capabilities of BTEX using a strain isolated from soil in oil-contaminated areas.

#### MATERIALS AND METHODS

First, 5 grams of soil were mixed with 25 milliliters of sterile distilled water. Then, one milliliter of the supernatant was enriched with 1% BTEX. After 14 days, this mixture was transferred onto the solid medium, and bacteria gram-positive, bacilli-shaped and large with slimy colony was isolated. Then, this isolate was incubated for an additional 14 days in a mineral medium containing 1% BTEX as the sole carbon source. On the 14<sup>th</sup> day of cultivation, Gas chromatography–mass spectrometry analysis was performed to assess the BTEX consumption. Additionally, molecular identification for this isolate was conducted using bacterial general primers.

#### RESULTS AND DISCUSSION

GC-MS results showed that this bacterium reduced benzene, toluene, ethylbenzene, ortho-xylene and para xylene by 98.2, 98.79, 99.54, 99.72, and 99.83%, respectively. This strain consumed all five compounds of BTEX at a rate of more than 98%. Also, this strain has the ability to grow in the presence of 3% BTEX at a temperature of 20 degrees Celsius. Therefore, this bacterium is a suitable option for the biological treatment of underground polluted water. Molecular identification of this isolate showed that this strain is 98% similar to *Bacillus haynesii*.

#### CONCLUSION

BTEX compounds are very toxic, in order to remove these compounds, we must search for capable strains that they have the ability to grow on pollutants and can bioremediate in different environmental conditions.

**Keywords:** Contaminant, Bioremediation, Aromatic hydrocarbons, GC-MS, 16s rRNA gene

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Screening and identification of Polyhydroxybutyrate (PHB) producing bacteria from isolated *Azotobacter* strains in agricultural soils

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Synthetic plastics are non-degradable and cause waste disposal problems leading to environmental pollution. Bioplastics, Polyhydroxyalkanoates (PHAs), are considered as suitable alternative for petroleum-derived synthetic plastics because of their similar physical and chemical properties. PHAs are biodegradable polymers produced by many microorganisms, of which Polyhydroxybutyrates (PHBs) are the best known PHAs. In the present study an attempt was made to isolate efficient PHBs producing bacteria like *Azotobacter* strains from soil samples.

#### MATERIALS AND METHODS

For this purpose, soil samples were collected from agricultural fields in West Azerbaijan, North Khorasan, Golestan, Mazandaran and Lorestan provinces. In order to isolate *Azotobacter* strains, soil samples were enriched in Winogradsky's nitrogen-free liquid medium for 3-7 days. Consequently, turbidity coming from all the tubes showing growth was streaked onto Winogradsky agar medium. All isolates were purified by cultivating on this medium. Further, the exact species level identification of *Azotobacter* was done by various morphological and biochemical tests. Finally, the purified bacterial isolates were screened to detect PHAs accumulation with the Sudan black B & Nile blue A staining method.

#### RESULTS AND DISCUSSION

In this research, about 24 *Azotobacter* isolates were obtained from 17 different soil samples. The Morphological and Biochemical characteristics of *Azotobacter* isolates were recorded as: Gram-negative, oval/rod shape, milky white and slimy colonies, yellow/brown-dark colonies in old cultures and non-diffusible pigments. In addition all bacterial isolates have the capacity to produce oxidases and catalases, cyst forming ability, Carbohydrates utilization and containing motile and non-motile ability. As a result, among the 24 bacteria, only 9 showed Sudan Black positive. This showed distinct black granules when viewed under a microscope. Nile blue A fluorescent staining further confirmed the presence of PHB granules. The cells that demonstrated a bright yellowish-orange color under a fluorescence microscope were confirmed as PHBs positive isolates.

#### CONCLUSION

The search for promising strains of PHAs producers is a continuous process and development of efficient PHAs producing bacteria are the need of the hour. On the basis of data obtained in the present work it can be concluded that *Azotobacter* strains isolated can be employed in the production of PHAs.

**Keywords:** Bio-plastics, Polyhydroxybutyrate, *Azotobacter*, Sudan black B, Nile blue A.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The inhibitory effect of *Lactobacillus rhamnosus* on p38-MAPK pathway activation and the expression of IL-17A

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Multiple sclerosis (MS) is one of the most common autoimmune disorders of the central nervous system (CNS), characterized by excessive immune cell infiltration, degeneration of the myelin sheath, and apoptosis of neuronal cells. It is now known that inflammation significantly contributes to the pathogenesis of the disease. Several lines of evidence have demonstrated that the p38 mitogen-activated protein kinase (MAPK) pathway regulates the immune cell response and programmed cell death and cell survival. Also, it has been shown the activation of the p38-MAPK pathway participates in the pathophysiology of various neurodegenerative disorders, such as Parkinson's disease and MS. In the present study, we examined the effect of *Lactobacillus rhamnosus* on the expression of p38-MAPK and Interleukin-17A (IL-17A) in a murine model of MS called experimental autoimmune encephalomyelitis (EAE).

### MATERIALS AND METHODS

Mice were immunized with myelin oligodendrocyte glycoprotein 35-55 (MOG35-55), followed by the injection of pertussis toxin to induce paralysis EAE mice. Following the induction of EAE, the animals received *Lactobacillus rhamnosus* by gavage when the early clinical signs of EAE began to appear. ELISA and real time pcr was done for expression of p38-MAPK and IL-17A.

### RESULTS AND DISCUSSION

The results showed that the administration of *Lactobacillus rhamnosus* led to a noticeable reduction in the clinical score of EAE mice. Moreover, the protein expression of the phosphorylated form of p38-MAPK was significantly diminished in the sacral region of the spinal cord of EAE mice receiving *Lactobacillus rhamnosus* when compared with EAE mice receiving no treatment (control group). The real-time PCR technique indicated that *Lactobacillus rhamnosus* lowered the expression of IL-17A in the spinal cord of EAE mice compared with the control group.

### CONCLUSION

Finally, it seems that probiotics, such as *Lactobacillus rhamnosus*, could be used as an alternative therapeutic option for alleviating neurological diseases in which the p38 MAPK signaling pathway is involved.

**Keywords:** Multiple sclerosis; Experimental autoimmune encephalomyelitis; p38-MAPK; Interleukin-17A; *Lactobacillus rhamnosus*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluating the bactericidal Activity of a disinfectant agent on surfaces

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

Hospital-acquired infections (HAIs) are a widespread issue, particularly in developing countries, meanwhile Surfaces play an important role in transmission of diseases. The main purpose of infection control in hospitals is to prevent the spread of infections. Disinfection and sterilization are essential for preventing the transmission of Pathogenic microorganisms to patients. This article has emphasized the necessity of surface sterilization procedure. The chemical sterilizing agent used in study was Steril-C, which produces Peracetic acid (PAA). PAA is based on low-temperature sterilization technology. The aim of this study is assessment of disinfection effect of PAA on surface.

##### MATERIALS AND METHODS

This study was accomplished in order to evaluating disinfection activity of PAA for surfaces. *E.coli* is a major cause of nosocomial infections so in this study *E.coli* ATCC 25922 was used and prepared a working culture. Then the test suspension containing  $1.5 \times 10^8$  to  $5 \times 10^8$  cfu/ml of *E.coli* was made. The test suspension and interference substance and PAA was mixed to evaluate disinfection activity of PAA. To negative control of test suspension, distilled water was used instead of PAA. For preparing validation suspension, test suspension and diluent was mixed and then cultured.

##### RESULTS AND DISCUSSION

PAA disinfectant solution was able to reduce more than 6 log number of microorganisms and there were no visible colonies on the blood agar culture medium. PAA is a powerful disinfectant with a wide spectrum of antimicrobial activity. Previous studies demonstrated that the residual bacterial number of the PAA-disinfected endoscope was significantly lower than Glutaraldehyde and Ortho-Phthalaldehyde disinfected endoscopes.

##### CONCLUSION

Peracetic Acid is able to destroy all the microorganisms in this suspension, thus this agent can be used as a high-level disinfectant with powerful effect. Therefore Peracetic Acid can be appropriate to disinfection of medical surfaces.

**Keywords:** PAA, disinfectant, surface, *E.coli*, infection control

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Efficient stabilization of the *Mycobacterium tuberculosis* recombinant antigens for *ex-vivo* usages

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

One of the main goals of TB research is to identify antigens that have the ability to induce cellular and/or humoral immunity. These antigens can be used in diagnostic reagents or vaccine design. Therefore, the stability of these recombinant antigens is critical. The addition of additives to Mtb recombinant antigens during storage is critical for increasing its stability and prevention of degradation. In this study, the effect of additives in different concentrations on the degradation of *Mycobacterium tuberculosis* antigens include CFP-10, ESAT-6 and TB7.7 at different times and temperatures was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

### MATERIALS AND METHODS

Mtb antigens were cloned, and expressed in *E. coli* BL21. Several formulations were prepared from the combination of additives. These formulas were combined with antigens in certain concentrations and antigens degradation was assessed at different times and temperatures by SDS-PAGE method. Also, the lyophilization of antigens, the lysis rate of red blood cells (RBC) and the cytotoxicity of white blood cells (WBC) in combination with each of the formulas were investigated.

### RESULTS AND DISCUSSION

The results showed that additives such as sucrose and mannitol in concentrations of 1.5% and 0.5% respectively in combination with antigens for 9 months at -20 °C had the ability to preserve antigens compared to the control sample. Also, the comparison of the formulas showed that the presence of gelatin increases the stability of antigens at different temperatures, including 25 °C. However, gelatin and glycerol increased the lysis of red blood cells in the tests. The results showed that the presence of additives include sucrose, mannitol, glycine and polyvinyl alcohol are necessary for better lyophilization of antigens.

### CONCLUSION

Mtb recombinant antigens stabilization using chemical additives inhibits antigens degradation, leading to increased antigens stability and purification efficiency.

**Keywords:** *Mycobacterium tuberculosis*, recombinant proteins, Lyophilization, proteins stability

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Human Microbiome and Allergic Diseases

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

The microbiome consists of many microorganisms, including bacteria, fungi and viruses, as well as their genomic elements. There is evidence that the human microbes residing in the airways, gastrointestinal tract and skin play an important role in normal health and disease states. Mucosal surfaces that line our gastrointestinal tract are continuously exposed to trillions of bacteria that form a symbiotic relationship and impact host health and disease. These data showed interpersonal differences in the composition of the microbiome and these differences suggest a link between the microbiome, the immune modulation, and the pathogenesis of allergic diseases. This research is particularly relevant in paediatrics, since allergic diseases are constantly increasing and there is evidence in the paediatric age that shows that the composition of the microbiome in the foetal and neonatal period plays a key role in the development of the immune system.

##### MATERIALS AND METHODS

A study of compared the microbiome of 46 children allergic to cow's milk proteins and 46 children no allergic. By analysing their gut microbiome: allergic subjects showed a greater variety of bacterial species compared to healthy subjects. At the end of 6 month hydrolysed milk diet: The microbiome of allergic subjects has showed changes such as a reduction in bifidobacteria and increase in lactobacilli compared to healthy subjects.

##### RESULTS AND DISCUSSION

The emerging view of asthma is one consistently related to inappropriate microbial community function in both the airway and gastrointestinal tract. This opens up the possibility that strategies to rationally manipulate microbiota at these sites may represent a novel approach to disease prevention.

##### CONCLUSION

In conclusion, Understanding the biology of the microbiome and how it interacts with the host to maintain gut homeostasis will be key to developing.

**Keywords: Allergy; Microbiome; Diseases.**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Cyclomodulin encoding genes in *E.coli* from CRC patients

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Colorectal cancer (CRC) is one of the most common types of cancer in the world. There are several factors involved in the creation of CRC, such as age, genetic factors, unhealthy behaviors, microbial factors, and inflammatory bowel diseases. *E. coli* is one of the members of intestinal microflora that may play a prominent role in CRC. In some *E. coli* strains, there are a set of virulence factors such as cyclomodulins (colibactin and cytotoxic necrotizing factors) and siderophores (such as aerobactin) that can affect the balance of intestinal microbiota and also cause changes in intestinal cells, leading to gastrointestinal malignancies or CRC, over time. The aim of this study was to investigate the frequency of *E. coli* isolates carrying cyclomodulins and aerobactin genes, in fecal specimens of patients and healthy individuals.

#### MATERIALS AND METHODS

In this study, 134 fecal samples including samples from patients with inflammatory bowel disease (IBD) or CRC (n=67) and those from healthy ones (n=67) were collected. The patients were selected from individuals whose disease was diagnosed for the first time and had not received any treatment (new cases). Also, they had not taken any antibiotic in the last two weeks before sampling. Isolation and identification of *E. coli* isolates was performed by culture methods and biochemical tests. Genomic DNA was used for looking for the presence of *clbN*, *cnf1* and *iutA* by PCR assay.

#### RESULTS AND DISCUSSION

The frequency of studied genes is shown in Table 1. There were significant differences between the presence of all studied genes in *E. coli* isolates of CRC patients with IBD and healthy ones (Table 1).

Table 1: Frequency (%) of some virulence factors encoding genes in fecal *E.coli* isolates from patients with CRC and IBD and healthy individuals

Virulence genes	CRC (n=21)	IBD (n= 46)	Control (n=67)	p- value
<i>clbN</i>	4 (19.4)	2(4.3)	3(4.4)	0.068*
<i>cnf1</i>	4 (19.04)	1(2.1)	3(4.4)	0.034**
<i>iutA</i>	10 (47.6)	19 (41.3)	12 (17.9)	0.005**

CRC: colorectal cancer; IBD: inflammatory bowel disease, \*Confidence interval of 93%; \*\*confidence interval of 95%

#### Conclusion

The presence of *E. coli* isolates containing cyclomodulins and aerobactin encoding genes, may be proposed in the development of gastrointestinal malignancies and may be used as markers for prediction of CRC. In addition, these factors may be targeted for therapeutics purposes in further researches. However, definite decision needs further studies.

**Keywords:** *E.coli*; cyclomodulin; *pks*; siderophore; colorectal cancer

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Polyphasic Identification of a Novel Halophilic Archaeon Isolated From a Solar Saltern in Spain

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Extremely halophilic archaea belonging to domain Archaea, represent a unique group of microorganisms that have been isolated from solar salterns with very high salinity. During a survey on SA` Vall solar saltern (Mallorca, Spain), a haloarchaeal strain designated AV220B87<sup>T</sup> was isolated from a brine sample and found to be closely related to members of genus *Halohasta*.

### MATERIALS AND METHODS

Phenotypic characterization of the strain AV220B87<sup>T</sup> was performed according to proposed minimal standard protocols. Polar lipids were analyzed using thin-layer chromatography. 16S rRNA gene sequence analysis was performed by cloning PCR products and EzBioCloud server was used for sequence similarity analysis. The phylogenetic position was determined in trees reconstructed using neighbor-joining, maximum likelihood and minimum evolution algorithms.

### RESULTS AND DISCUSSION

Results show that cells of this strain are non-motile and gram-stain-negative. Optimal NaCl, Mg<sup>2+</sup>, pH and temperature for growth are 3.5 M, 0.1 M, 7 and 40 °C, respectively. Reduction of nitrate to nitrite and indole production is negative. Cells hydrolyze gelatin while casein, starch and tween 80 hydrolysis is negative. No growth observed with DMSO and L-arginine. Furthermore, the strain AV220B87<sup>T</sup> is sensitive to: novobiocin, rifamycin B, Nitrofurantoin. The total polar lipid pattern include phosphatidylglycerol, phosphatidic acid, phosphatidylglycerol sulfate, phosphatidylglycerol phosphate methyl ester with some unknown phospholipids and glycolipids. The 16S rRNA gene sequence of strain AV220B87<sup>T</sup> share the highest sequence similarity with members of *Halohasta* (*Halohasta litorea* R30<sup>T</sup> and *Halohasta litchfieldiae* tADL<sup>T</sup>, 97.8% and 97.1% respectively). Moreover, based on the neighbour-joining tree, strain AV220B87<sup>T</sup> constitute a distinct monophyletic branch.

### CONCLUSION

The sequence similarity of strain AV220B87<sup>T</sup> to the most closely related genera and phylogenetic trees indicate that this strain can be represented as a novel species in the genus *Halohasta* within the family *Haloferacaceae*.

**Keywords:** Solar Salterns, Halophilic Archaea, Polyphasic Identification

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Biofilm-associated genes correlates with biofilm phenotypes in *Pseudomonas aeruginosa* treated with nano-Fe<sub>3</sub>O<sub>4</sub> and nano-Ag

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The opportunistic human pathogen *Pseudomonas aeruginosa* endorse biofilm-associated infections that enhanced resistance to antimicrobial agents. There are few effective treatments for *P. aeruginosa* biofilm-associated infections, necessitating novel therapeutic to target biofilm. The objectives of this study were to investigate anti-planktonic activity, ability to inhibit pre-formed biofilm, and to investigate the expression levels of biofilm-associated genes in pre-formed biofilms after (nano-Fe<sub>3</sub>O<sub>4</sub> and nano-Ag) treatments.

### MATERIALS AND METHODS

In this study, we monitored the anti-planktonic and anti-biofilm activities of nano-Fe<sub>3</sub>O<sub>4</sub> and nano-Ag against *P. aeruginosa* by minimum inhibition concentrations (MIC), minimum bactericidal concentration (MBC), crystal violet staining, XTT Assays and light microscopy using various microbiological protocols. We performed expression analysis of biofilm-associated genes in *P. aeruginosa* treated with various concentrations of nano-Fe<sub>3</sub>O<sub>4</sub> and nano-Ag based on the MIC by quantitative Real-time PCR method.

### RESULTS AND DISCUSSION

The nano-Fe<sub>3</sub>O<sub>4</sub> and nano-Ag represented great antibacterial properties at concentration of 50 and 1.625 µg/mL, respectively. The MBC values of nano-Fe<sub>3</sub>O<sub>4</sub> and nano-Ag were 100 and 3.125 µg/mL, respectively. The crystal violet and XTT assays on *P. aeruginosa* biofilm quantification demonstrated a significant reduction of biomass and metabolic activities in response to various concentrations (2× MIC, 1× MIC and ½× MIC) of nano-Fe<sub>3</sub>O<sub>4</sub> and nano-Ag. The resultant biofilms were viewed with light microscopy, which show a significant difference in biofilm density exposed to the nano-Fe<sub>3</sub>O<sub>4</sub> and nano-Ag as compared with untreated control. Moreover, the relative expression profiles of *PELA* and *PSLA* showed alterations in expression levels by the treatment with nanoparticles in a concentration dependent manner, which correlated with the biofilm phenotypes in *P. aeruginosa*.

### CONCLUSION

The potential of nano-Fe<sub>3</sub>O<sub>4</sub> and nano-Ag could reduce the expression of *PELA* and *PSLA* as important of biofilm-associated genes. Although more research is needed, the use of nano-Fe<sub>3</sub>O<sub>4</sub> and nano-Ag as a new therapeutics in *P. aeruginosa* infections is recommended.

**Keywords:** Biofilm; Nanoparticles; *PELA*; *PSLA*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The role of gut microbiome in hematopoietic stem cell transplantation (HSCT) patients

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### ABSTRACT

The role of the gut microbiome (GM) in the field of hematopoietic stem cell transplantation (HSCT) has been associated with promising results in the last decade. However, infections and graft-versus-host disease (GVHD) are the two major complications that contribute significantly to transplant-related mortality. A diverse microbiota is required to maintain host microbe homeostasis in various environmental changes because species-rich bacterial communities can compensate for those lost. In HSCT, along with microbiome diversity as an indicator of outcomes, some pathogenic bacterial species are increased in GVHD recipients such as *Enterococcaceae*, *Akkermansia muciniphila* and *Lactobacilles*. On the other hand, *Blautia* has been shown as a beneficial bacterial genus for HSCT, as well as the association of increased GVHD with a decrease in the abundance of some bacterial genera such as *Faecalibacterium*, *Bacteroides* and *Parabacteroides*. *Lachnospiraceae* and *Enterococcaceae* are essential for maintaining the integrity of the gastrointestinal tract and facilitating long-term immune system reconstruction after radiation exposure. In addition, the disturbance in the intestinal microbiota is associated with major complications in allogeneic hematopoietic stem cell transplantation (allo-HSCT), and various strategies have been developed to reduce dysbiosis and related complications. Fecal microbiota transplantation (FMT) involves the injection of fecal material from a healthy donor to restore impaired intestinal homeostasis and can be applied to HSCT.

**Keywords:** Gut Microbiome, Hematopoietic Cell Transplantation (HSCT)

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of antibacterial efficacy of *Syzygium cumini* leaf extract against *Vibrio cholerae*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Cholera is an acute diarrheal infection with epidemic potentials, caused by *Vibrio cholerae*. In the recent years, *V. cholerae* strains have emerged as a multidrug resistance of enteropathogenic bacteria. The antibiotics could be ineffective against infections causing multidrug resistant enteric pathogenic bacteria and phytodrug could be helpful for the control of these infections. *Syzygium cumini* is a tropical medicinal fruit show various therapeutic applications as antibacterial and antidiarrheal effects. The aim of current study was to investigate the effect of *S. cumini* leaf extract against Non-O1/Non-O139 *V. cholerae* clinical isolates.

### MATERIALS AND METHODS

The antibacterial activity of aqueous and ethanolic leaf extracts of *S. cumini* were evaluated against Non-O1/Non-O139 *V. cholerae* clinical isolates. Three clinical isolates (completely susceptible, antibiotic resistant, MDR) of *V. cholerae* were analyzed for assessment of antivibriocidal activity of *S. cumini* extracts. Minimum inhibitory concentration (MIC) of extracts was determined by the micro-broth dilution method.

### RESULTS AND DISCUSSION

The aqueous & ethanolic leaf extracts of *S. cumini* were able to inhibit the growth of Non-O1/Non-O139 *V. cholerae* clinical isolates with a noteworthy MIC value of 7500 and 2500 µg/ml for susceptible strain, 500 and 2000 µg/ml for antibiotic resistant strain and 1000 and 2000 µg/ml for MDR strain, respectively. In this study, leaf extracts of *S. cumini* was exhibited potential activity against all susceptible, antibiotic resistant and MDR isolates of *V. cholerae*.

### CONCLUSION

Now a days, increased antimicrobial resistance is the cause of severe infections and increased mortality, thus safer novel antibacterial agents are required. In present study, the leaf extracts of *S. cumini* showed antivibriocidal effects especially against MDR clinical isolate. This outcome is remarkable considering that leaf extract of *S. cumini* seem to be promising as a complementary medicine to control of cholera infection, because of its potential for inhibiting MDR *V. cholerae* and its less cytotoxic properties.

**Keywords:** *Vibrio cholerae*, *Syzygium cumini*, Multidrug resistance.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Microbial induced calcite precipitation (MICP) potential of ureolytic *Bacillus* sp. isolated from the soil of eroded ecosystems for stabilizing and improving the fertility of eroded soils

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

The loss of soil from lands due to erosion has a negative effect on ecosystems and food security. *Bacillus* due to high catabolic capability is an appropriate candidate for application in biocementation process. The aim of this study is isolate and characterize *Bacillus* sp. with biocementation capability from various ecosystems.

#### MATERIALS AND METHODS

The isolates were separated from 400 samples, and characterized by biochemical and molecular methods include the amplification and sequencing analysis of *gyrA* and 16S rRNA genes. Growth in presence of urea, in different salinity, pH and temperature, also scanning electron microscope (SEM), X-ray diffraction (XRD) and wind tunnel analysis were applied to determine biocementation ability.

#### RESULTS AND DISCUSSION

A total number of 195 isolates were recovered from environmental samples, of which 25 isolates (12.82%) were identified as urease-positive *Bacillus* which belonged to 10 species consisting of *B. subtilis* 5 strains (20%), *B. vallismortis* and *B. seohaeanensis* 4 strains (16%) each, *B. mobilis*, *B. pseudofirmus*, *B. cohnii*, *B. cereus*, *B. alkalinitrilicus* 2 strains (8%) each, and *B. sphaericus* and *B. megaterium* 1 strains (4%) each. Moreover, 15 urease- positive isolates (7.7%) belonging to *Ralostenia*, *Actinomycece* and *Halomonas* genera were identified. Optimum conditions for microbial induced calcite precipitation (MICP) by isolates are 30°C, pH 9 and 6% salinity. The highest rate of calcium carbonate formation and urease activity recorded in *B. subtilis* with 24.15 mg/mL of calcium carbonate and 4.40 × 10<sup>3</sup> unit/L of urease, followed by *B. mobilis* and *B. alkalinitrilicus* with 22.85 mg/mL of calcium carbonate and 3.93 × 10<sup>3</sup> unit/L of urease. After MICP the lowest soil loss ratio at a flow rate of 90 km/h, was observed in *B. subtilis* 100-fold reduction, followed by *B. seohaeanensis*, *B. cereus*, *B. vallismortis*, with 90,85,80 folds reduction respectively.

#### CONCLUSION

Results indicate that the diversity of *Bacillus* sp. offers the potential ability for adaptation to harsh and untapped environment, also showed that the use of MICP on the soil surface can have a very significant role on reducing soil losses due to wind erosion.

**Keywords:** MICP, Urease Positive *Bacillus*, Soil Erosion, Biocementation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The anti-biofilm effect of extracellular vesicles extracted from *Holothuria Parva* tissue on *Staphylococcus aureus*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Staphylococcus aureus* is a gram-positive bacterium that causes a wide range of clinical diseases. Infections caused by this pathogen are common in both community-acquired and hospital-acquired environments. Resistance to antibiotics has become very common in this bacterium, and in the past years, it has undergone significant . In general, biofilm formation has been challenging in the field of medicine because it interferes with ongoing medications and is associated with various diseases. Biofilms increase antibiotic resistance in the way that the dense extracellular matrix and the outer layer of the cell protect the entire biofilm community. Various anti-biofilm compounds have been used by researchers to inhibit and disturb the biofilm structure. The marine environment is an exceptional reservoir of natural products, many of which have structural properties not found in terrestrial natural products. The sea cucumber is a valuable aquatic organism that has many uses in the food, pharmaceutical and medical industries. It is well known that sea cucumbers have beneficial effects on human health and can be used for medicinal purposes. In this study, *Holothuria Parva* extracellular vesicles were used as one of the anti-biofilm substances.

### MATERIALS AND METHODS

*H. Parva* sea cucumbers were collected from the Persian Gulf, coasts of Bushehr province. The extracellular vesicles were extracted from their tissue. Cultivation and recovery of *Staphylococcus aureus* ATCC 6538 was performed on BHI agar. Kirby-Bauer disk diffusion susceptibility test and broth microdilution assay (MIC) of sea cucumber extracellular vesicles was used for antibacterial activity. Also, the effect of sea cucumber extracellular vesicles on *S. aureus* ATCC 6538 biofilm with Minimum biofilm inhibitory concentration (MBIC) was investigated by crystal violet biofilm assay method. Different concentrations of extracellular vesicles (0.15, 0.31, 0.62, 1.25, 2.5, 5 mg/ml) were tested on the bacterium.

### RESULTS AND DISCUSSION

The results showed that *H. Parva* extracellular vesicles had no antibacterial effect on *S. aureus* ATCC 6538 and finally, anti-biofilm effect was not seen.

### CONCLUSION

It is hoped that the anti-biofilm effect of extracellular vesicles will be investigated by a researcher on the other bacteria in future studies.

**Keywords:** Anti-Biofilm, Persian Gulf Sea Cucumber, Extracellular Vesicles, *S. Aureus* ATCC 6538

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular detection and identification of hemotropic *Mycoplasma* species in dogs, cats, and their ectoparasites in Iran

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Hemotropic *Mycoplasma* species are vector-borne bacteria that attach and grow on the surface of erythrocytes in various mammals, yet reports of canine and feline hemoplasmosis in Iran are scarce. The aim of this study was molecular detection and identification of hemoplasmas in the blood of dogs and cats from different regions of Iran.

### MATERIALS AND METHODS

From December 2018 to February 2021, a total of 370 dogs from Hamedan, Kermanshah, Yazd, Amol and Ahvaz, and 361 cats from Tehran, Hamedan, Kermanshah, Yazd, Kerman and Mashhad were examined clinically, their bodies were searched for collection of ectoparasites, and cephalic or saphenous blood specimens were collected. Genomic DNA was extracted from blood and ectoparasite specimens and the presence of hemotropic *Mycoplasma* DNA was detected using group-specific, and identified using species-specific conventional PCRs detecting *Mycoplasma haemocanis* (Mhc), *Candidatus Mycoplasma haematoparvum* (CMhp), *Candidatus Mycoplasma haemominutum* (CMhm), *Mycoplasma haemofelis* (Mhf) and *Candidatus Mycoplasma turicensis* (CMT) followed by Sanger sequencing. Correlation of infection and risk factors (geographical area, keeping condition, body condition, sex, age, infestation with ectoparasite) were analyzed.

### RESULTS AND DISCUSSION

In group-specific PCR, 210 dogs (56.7%) and 57 cats (15.7%) tested PCR-positive for hemotropic *Mycoplasma*. Species-specific PCR and sequencing revealed infection with Mhc in 17.8%, with CMhp in 7.02% and with both parasites in 31.9% of dogs. In cats, positivity rates of 10.2% for CMhm, 2.2% for Mhf, 0.9% for CMT and 2.50% for both CMhm and Mhf (co-infection) were recorded. Flea infestation, poor body condition, and being older than 3-years-old correlated with hemoplasmosis. Ectoparasites collected from dogs included *Ctenocephalides canis* and *Pulex irritans* fleas, *Rhipicephalus sanguineus* sensu lato ticks, *Heterodoxus spiniger* lice and *Hippobosca longipennis* keds in which DNA of hemoplasmas were detected only in fleas i.e. Mhc in *P. irritans*, CMhp in *P. irritans* and *C. canis*, and co-infection with Mhc an CMhp in *C. canis* collected from dogs. No ectoparasite was collected from cats.

### CONCLUSION

To our knowledge, this is the first large-scale molecular epidemiology study of canine and feline hemoplasmosis in Iran. Considering the high prevalence of canine and feline hemoplasmosis all over the country including potentially zoonotic CMhp in dogs, and CMhm in cats, effective ectoparasite control strategies, regular examination of dogs and cats, successful chemoprophylaxis and public awareness strategies are advocated.

**Keywords:** blood, cat, dog, ectoparasite, PCR, hemoplasma, one health

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibacterial and anticancer effects of silibinin functionalized silica-coated Fe<sub>3</sub>O<sub>4</sub> nanocomposite on *Pseudomonas aeruginosa* isolates and HepG2 cell line

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Silibinin, a polyphenolic flavonolignan from *Silybum marianum* seeds, has anti-bacterial, anti-oxidant, and anti-cancer activities. This study aimed to design and synthesize a magnetic nanocarrier to deliver silibinin to cancer and bacteria cells.

### MATERIALS AND METHODS

physicochemical measurement of silibinin functionalized silica-coated Fe<sub>3</sub>O<sub>4</sub> nanocomposite were evaluated by FESEM, FT-IR, VSM, and XRD tests. *Pseudomonas aeruginosa* isolates were treated with ciprofloxacin (sub-MICs) and a combination of ciprofloxacin with a Fe<sub>3</sub>O<sub>4</sub>@SP@silibinin. Furthermore, the cytotoxic effects of Fe<sub>3</sub>O<sub>4</sub>@SP@silibinin on HepG2 cells were investigated by MTT assay.

### RESULTS AND DISCUSSION

The physicochemical analyses have confirmed the synthesis of the proposed structure for silibinin nanocomposites. Additional measurements have also been taken to determine the particle size and paramagnetic properties. The antibacterial activity of ciprofloxacin was increased in combination with Fe<sub>3</sub>O<sub>4</sub>@SP@silibinin. The expression of porin genes in *P. aeruginosa* was increased while the expression of biofilm and efflux pump genes was decreased. MTT assay analysis illustrated that Fe<sub>3</sub>O<sub>4</sub>@SP@silibinin in a dose- and time-dependent manner inhibited HepG2 cell proliferation and migration.

### CONCLUSION

It seems that Fe<sub>3</sub>O<sub>4</sub>@SP@silibinin can be used in biomedical applications, especially in antimicrobial and cancer therapeutics in the future.

**Keywords:** *Pseudomonas aeruginosa*, Biofilm, HepG2, silibinin, magnetic nanoparticles

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Harvesting yeast (*Saccharomyces cerevisiae*) at different physiological phases significantly affects its functionality in bread dough fermentation

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Yeast cells are also partly responsible for bread flavor and may affect dough rheology. Entrance into the diauxic shift is accompanied by an increase in storage molecules such as glycogen and trehalose. As the cells approach the stationary phase, different enzymes and storage carbohydrates accumulate. To this end, yeast was harvested at seven distinct points during growth, characterized and subsequently used in the production of dough.

### MATERIALS AND METHODS

The effect of harvesting yeast at seven different points, its characteristics on the growth of fermentation performance, metabolite production and the effect on important fermentation indicators were investigated. yeast was harvested at seven distinct points during growth, characterized and subsequently used in the production of dough. The supernatants of fermented growth media were dried in an oven at 100 C for 24 h. Total gas production and gas retention are measured with a pressure sensor through direct and indirect cycles, respectively. During the direct cycle, the changes in the gas pressure inside the fermentation tank are being recorded and this leads to the total gas production curve. This gives the total gas retention of dough during the fermentation process.

### RESULTS AND DISCUSSION

The first three points showed little succinic acid production while the cells harvested and in the next four points showed up to fourfold higher succinic acid production. This threshold might be variable for different dough samples based on the fermentation speed of yeast. The combination of produced metabolites by the yeast during dough fermentation, and the fermentation rate of yeast at different harvest points might be the key determining factors on gas holding capacity.

### CONCLUSION

The results demonstrate the importance of yeast harvest time on the subsequent dough fermentation capacity of yeast, including fermentation rate and metabolite production. Hence, we postulate that differences in production of metabolites such as ethanol, acetic acid and succinic acid by the yeast during dough fermentation might influence dough extensibility, and therefore affect the gas holding capacity of fermenting dough.

**Keywords:** *Saccharomyces Cerevisiae*, Diauxic Shift, Fermentation Parameter

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The effect of the mixture of lactic probiotics on the antioxidant and total oxidant status of the kidney tissue of rats exposed to gentamicin

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Gentamicin is one of the aminoglycoside antibiotics that is still used in the treatment of infections, especially against Gram-negative bacteria, but one of its main side effects is nephrotoxicity. This study aims to investigate the probiotic mixture (*Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus heloticus*) on the antioxidant and oxidant status of the total kidney tissue of rats exposed to gentamicin.

### MATERIALS AND METHODS

In this experimental study, 21 adult male mice with a weight of 200-250 grams were divided into three control groups, receiving gentamicin (100 mg/kg), receiving gentamicin (100 mg/kg) + receiving probiotic mixture (10<sup>9</sup> CFU/MI). Intraperitoneal injection of gentamicin was performed for 8 days and gavage of probiotics was performed for 30 days. After the treatment period, the kidney tissue was extracted to check the antioxidant capacity and antioxidant status. Data analysis in different groups was done with SPSS software and one-way variance statistical test and P<0.05 was considered significant.

### RESULTS AND DISCUSSION

The results showed a significant change in the level of antioxidants; It showed a decrease in the total antioxidant capacity of TAC and an increase in the total antioxidant status of TOS in the group receiving gentamicin (P<0.001). Also, the change in the level of antioxidants TAC and TOS in the group treated with probiotic mixture was shown to be significant compared to the group receiving gentamicin(P<0.05).

### CONCLUSION

Based on the obtained results, the protective effect of the probiotic mixture on the induced toxicity of gentamicin on the antioxidant capacity of the kidney tissue was shown. This effect can be by reducing the oxidative stress caused by free radicals and reducing the amount of lipid peroxidation caused by gentamicin.

**Keywords:** Probiotic, Gentamicin, Oxidative stress indicators, Rat

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation of the resistance pattern of anti-urinary tract infection antibiotics among outpatients of Central Laboratory of Tabriz, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Urinary Tract Infection (UTI) is one of the most common infectious diseases known in outpatients and hospitalized patients. Nowadays, with the increasingly arbitrary use of antibiotics around the world, antibiotic resistance patterns are constantly changing. Therefore, in this study, we analyze the resistance patterns of the antibiotics which are usually the first treatment for UTIs to identify the most effective antibiotic against UTI.

### MATERIALS AND METHODS

In this cross-sectional study, urine samples of 9,000 patients who attended the central laboratory of Tabriz, Iran (from February to July 2023) were analyzed for the isolation of microbial species by standard methods. Specific differential diagnosis tests were performed to detect the type of bacteria then a CLSI antibiogram test was employed using the disk diffusion method to check the antibiotics resistance pattern.

### RESULTS AND DISCUSSION

All of the 9,000 urine samples were cultured and 392 of them turned out to be positive. Among the positive samples, *Escherichia coli* and *Klebsiella pneumoniae* with a prevalence of %80.35% and 12.75% were the most common causes of UTIs in our sample size, respectively. Other pathogens such as *Citrobacter freundii*, *Enterococcus faecalis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Staphylococcus saprophyticus*, *Pseudomonas*, and *Beta Hemolytic Streptococcus* had the lowest prevalence (6.88%). The ratio of UTIs in females was significantly higher (88.78%) than in males (11.22%). In our study, *Escherichia coli*, the most common cause of UTIs, showed the highest antimicrobial sensitivity to Nitrofurantoin (antibiotic resistance of 8.25%) and Gentamicin (antibiotic resistance of 12.69%) with the maximum resistance to Ampicillin (antibiotic resistance of 71.12%). Also, *Klebsiella pneumoniae*, the 2nd most common cause of UTIs in our sample size, showed the highest antimicrobial sensitivity to Amikacin (antibiotic resistance of 16.08%) and Gentamicin (antibiotic resistance of 22.12%) with the highest resistance to Ampicillin (antibiotic resistance of 94.03%). Subsequently, the results of our study showed that both pathogens, *Escherichia coli* and *Klebsiella pneumoniae*, has the highest resistance to Ampicillin and Cephalexin.

### CONCLUSION

According to this study, for emergency cases in which antibiogram tests are not ready, Nitrofurantoin and Amikacin are recommended as the best choice antibiotics to start the empirical treatment of UTI.

**Keywords:** Amikacin, Antibiotic resistance, *Escherichia coli*, *Klebsiella pneumoniae*, Nitrofurantoin, UTI

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Antimicrobial activity of two Iranian honeys against methicillin resistance *staphylococcus aureus* (MRSA)

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Eryngo honey originated from *Eryngium* L. (a genus of flowering plants belonging Apiaceae) is known as Zoll in Persian language and is one of the tastiest and medical kinds of honey produced in the heights of the mountainous regions of Iran. *Gossypium* L., one of the famous plant taxa in Malvaceae is the origin of another monofloral honey produced in limited region in the cotton fields of Iran and has some therapeutic properties. In the present study, antimicrobial activity of these two Iranian honeys was examined against Methicillin resistance *Staphylococcus aureus*.

#### MATERIALS AND METHODS

For antimicrobial activity assay, 100-25 % concentrations of Eryngo and Cotton honeys were prepared. Antibacterial activity of recruited honeys was examined against Methicillin resistance *Staphylococcus aureus* (ATCC 1826) by disk diffusion assay. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were determined by broth microdilution method. All tests were performed in triplicate.

#### RESULTS AND DISCUSSION

Disk diffusion assay showed that at concentration of 100%, Eryngo honey and Cotton honey created inhibition zones of 32 mm and 34 mm against MRSA, respectively. Both tested honey types, totally inhibited bacterial growth at the 100% and 75% concentrations. No antimicrobial effects were observed at concentrations of 50% and 25%.

#### CONCLUSION

Until now, Manuka honey originates from the manuka bush (*Leptospermum scoparium*), an endemic plant grown in New Zealand is the most famous honey with antimicrobial properties. Result of this pilot study showed the Eryngo and Cotton honeys have remarkable antimicrobial effect on MRSA. Further study on more antibiotic-resistance bacteria may suggest these Iranian honeys as good candidates for medical applications

**Keywords:** Honey, antimicrobial effect, MRSA

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of the azithromycin resistance gene *mph(A)*, in clinical isolates of *Pseudomonas aeruginosa*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Infections with *Pseudomonas aeruginosa* have become a real concern in hospital-acquired infections, especially in critically ill and immunocompromised patients. The major problem leading to high mortality lies in the appearance of drug-resistant strains. We collected a series of clinical samples from the hospital and performed a disk diffusion test to screen samples resistant to azithromycin. Then we extracted DNA from the samples of interest. Then we performed PCR to verify the presence or absence of the gene of interest.

*Pseudomonas aeruginosa* is a common encapsulated, gram-negative, aerobic-facultatively anaerobic, rod-shaped bacterium that can cause disease in humans. They are found everywhere, in nature and hospital environments (e.g., flowers, sinks, toilets, ventilators, and dialysis machines). Multidrug-resistant *Pseudomonas aeruginosa* are of great concern to healthcare systems worldwide because of their simultaneous resistance to different classes of antibiotics. Antibiotics are considered a serious threat to healthcare workers. Azithromycin is widely used to treat various infections in both children and adults. Azithromycin resistance caused by phosphotransferase is encoded in the *mph(A)* and *mph(B)* genes. This gene has been discovered and described in many bacterial species.

### MATERIALS AND METHODS

100 Clinical samples were collected from Motahari Hospital. Antibiotics used in disc diffusion were FOX, AZM, CAZ, TE, CTX, and IMP. Then we separated the resistant samples and extracted their DNA by boiling and set a PCR test for each sample.

### RESULTS AND DISCUSSION

Almost all samples showed resistance to *azithromycin*. After checking the PCR results on these resistant samples, observations showed that more than 50% of the samples had the desired gene, and the results were consistent with previous studies.

### CONCLUSION

The presence of the target gene in most of the samples is a confirmation of the importance of increasing the antibiotic resistance of this bacteria as one of the factors of hospital infections and studying the factors involved in this resistance to improve the treatment of patients.

**Keywords:** *Pseudomonas aeruginosa*, azithromycin, resistance gene

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# Isolation and identification of *Lactobacillus* from Gilan traditional yogurt and the effect of its extract on *p21*, *p53* and *casp3* signaling pathways in MCF-7 breast cancer cell line

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Today, probiotics are used as supplements and have been increasingly noticed in recent years. Several studies have shown the ability of probiotics to prevent and treat various diseases, like cancer. Probiotics exert their anti-cancer effects through anti-inflammatory, antioxidant, anti-angiogenic activities. Therefore, they can be proposed as a rational approach for the prevention and treatment of cancer, including breast cancer. Therefore, the focus of this study is to investigate the effects of probiotics isolated from dairy products on MCF-7 breast cancer cells.

### MATERIALS AND METHODS

Various yogurt samples using sterile saline and then cultured in MRS agar medium. Then *lactobacilli* are identified using microbial and biochemical tests. Then, with the help of 16srRNA *Lactobacillus* specific primers, DNA extraction and PCR are carried out to determine the identity. Then, the identified *Lactobacillus* extract was determined by the MTT assay in order to determine the best effective dilution on the cells. Then, the extract with appropriate dilution was applied on HT-29 cancer cells. RNA extraction was performed for three hours and six hours after the effect of the extract on cancer cells. Then cDNA synthesis was done using the kit according to the protocol. In order to perform Real time PCR, primer design was performed on *p21*, *p53* and *casp3* genes.

### RESULTS AND DISCUSSION

Local dairy samples were collected from different regions of Gilan province and *lactobacilli* were isolated using MRS-Agar medium. In order to molecularly confirm the isolated from local yogurt samples as probiotic strains, DNA extraction was done using a kit in order to determine the amount of apoptosis induced in MCF-7 cancer cell line by *Lactobacillus* extract, respectively for gene *p21* equal to 2.77 and 2.42 for *p53* gene and 2.32 for *casp3* gene and 1 for GAPDH control gene. According to the results, the effect of *Lactobacillus* extract on MCF-7 cancer cells increases the expression of all three genes, which indicates an increase in apoptosis.

### CONCLUSION

The results of this study showed that by adding *lactobacillus*, the expression of *p21*, *p53* and *casp3* genes increases, which by activating the apoptosis pathway leads cancer genes to programmed death and can be used as a complementary treatment.

**Keywords:** *Lactobacillus*, traditional Gilan yogurt, *p21*, *p53* and *casp3*, MCF-7, breast cancer

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# Investigating the effects of *Lactobacillus* isolated from traditional dairy of Gilan province and its extract on the expression of *AKT*, *mTOR*, *PTEN*, *CASP3* genes in HT-29 clone cancer cells

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Treating diseases with the help of microorganisms is a new and developing method that uses beneficial bacteria as therapeutic agents in immune disorders and infectious diseases. Probiotics are non-pathogenic microorganisms that, when prescribed in appropriate amounts, can prevent or improve some diseases by improving the health status. Therefore, the aim of this study is to investigate the effects of probiotics isolated from dairy products on HT29.

### MATERIALS AND METHODS

Dairy samples from different regions of Gilan province were randomly purchased and collected in sterile containers, then MRS broth was used to isolate *Lactobacilli*. Biochemical and microbial tests were used for the phenotypic identification of *Lactobacilli*. Also, for the genotypic identification of *Lactobacillus*, using specific primers for the molecular identification of 16SrRNA of *Lactobacillus*, DNA extraction kit and PCR of *Lactobacillus* bacteria were used to identify them. Sequencing was done. After preparation and cultivation of HT-29 cells, HT-29 were treated with *Lactobacillus* extract by MTT assay. HT-29 cells were treated with *Lactobacillus* extract identified by MTT assay in different dilutions and the best effective dilution on the cells was determined. Finally, the expression of *AKT*, *mTOR*, *PTEN* and *CASP3* genes in cancer cell line was investigated by Real Time PCR.

### RESULTS AND DISCUSSION

Dairy samples including cow's milk, cheese and local yogurt were collected from different regions of Gilan province. And using MRS-Agar medium, *Lactobacilli* were isolated. DNA extraction was done using the kit. The results of investigating the treatment of HT-29 cells with *Lactobacillus* extract by MTT assay. First, the range of IC<sub>50</sub> was determined. HT29 cancer cells were treated with *Lactobacillus helveticus* extract at different concentrations of 0.5, 1, 1.5 and 2 µg/ml using the MTT test within 24 hours, and the results of decreasing cell viability at high concentrations were reported as 92.24, 85.24, 67.24 and 55.24, respectively. Also, the results showed that the extract of *Lactobacillus helveticus* bacteria at a concentration of 2 µg/ml has a 50% killing effect. In order to determine the amount of apoptosis induced in HT-29 cancer cell line by the extract of *Lactobacillus helveticus* bacteria, it was recorded as 2.47 for the *CASP3* gene, 0.76 for the *AKT* gene, 2.43 for the *PTEN* gene, and 0.98 for the *mTOR* gene, and this value was also recorded as 1 for the GAPDH control gene. According to the results of the effect of *Lactobacillus helveticus* extract on HT29 cancer cells, the expression of both genes increased and this effect was more on the expression of *fas* gene, which indicates an increase in apoptosis.

### CONCLUSION

The results of this study showed that *Lactobacillus*, especially *Lactobacillus helveticus*, has a high potential to prevent cancer and can be used as an adjuvant treatment. They were also used in the dairy food industry to improve food quality.

**Keywords:** *Lactobacillus*, traditional dairy products of Gilan province, expression of *AKT*, *mTOR*, *PTEN*, *CASP3* genes, HT-29 cells

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of the effects of suspension formulation on the viability of probiotic *Lactobacillus* isolate in cryopreservation

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Probiotics are important starters in the food and pharmaceutical industries. Their long-term maintenance is an important factor. These bacteria can be stored for a long time using freezing techniques like freeze-drying. However, freeze-drying damages bacterial cells. Therefore, to protect the bacteria, it is necessary to use cryoprotectants to increase bacterial survival.

### MATERIALS AND METHODS

In this experiment, lactose, cellobiose, sucrose, trehalose, and maltose were used as cryoprotectants at concentrations of 8%, 16%, and 24%. After culturing the bacteria, the desired cryoprotectant at different concentrations was added to the bacteria, followed by freeze-drying. Subsequently, the viability of the bacteria after freeze-drying was assessed.

### RESULTS AND DISCUSSION

The protective role of disaccharides is to maintain the stability of the cell membrane during freezing by reducing the formation of intracellular ice. After comparing the effects of different concentrations of sugars on bacterial viability, there was no significant difference in the survival of bacteria at 8% sugar concentration. At the concentration of 16%, trehalose and sucrose showed the highest viability rates, while lactose and cellobiose showed the lowest viability rates. At the concentration of 24%, trehalose had the highest survival rate, while cellobiose and lactose had the lowest survival rates.

### CONCLUSION

Using lactose and cellobiose as cryoprotectants resulted in lower viability rates of bacteria compared to other sugars. Therefore, these two sugars are not suitable options as cryoprotectants. The highest survival rate was observed with trehalose and sucrose. Sucrose directly interacts with lipids and proteins, replacing the hydrogen bonds that normally exist in water. Trehalose, which has a high glass transition temperature ( $T_g$ ), can significantly reduce the formation of ice crystals, thereby reducing mechanical damage to the cell membrane. The protective role of trehalose may be due to its stabilizing capacity for proteins, which replaces water around polar molecules.

**Keywords:** probiotics, freeze-drying, cryoprotectant , disaccharide

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Detecting *gyrA* and *parC* mutations fluoroquinolone-resistant *Pseudomonas aeruginosa*

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

*Pseudomonas aeruginosa* as important nosocomial pathogen commonly associated with aquatic environments. Various mechanisms are involved in the development of resistance to multidrug-resistant *P. aeruginosa*. The aim of this study was to investigate the association of *P. aeruginosa* resistance to fluoroquinolones family antibiotics and mutation in two *gyrA* and *parC* genes.

### MATERIALS AND METHODS

A total of fifty-three clinical strains of *P. aeruginosa* analyzed in this study, were obtained from inpatients of Loghman Hospital of Tehran Province. Then Kirby-Bauer disk diffusion assay was used for antibiotic resistance. Fluoroquinolone-resistant was confirmed by determining ciprofloxacin and levofloxacin minimum inhibitory concentration using the E-test according to the manufacturer's instructions. Detection of *gyrA* and *parC* mutations was done by PCR-restriction Fragment Length Polymorphism (RFLP).

### RESULTS AND DISCUSSION

According to antibiotic susceptibility testing out of 53 isolates, 49 were resistant to ciprofloxacin (92.4%), 47 isolates to levofloxacin (88.6%), 46 isolates to imipenem (86.6%), 46 isolates to trimethoprim-sulfamethoxazole (86.6%), 42 isolates to gentamicin (71.1%), 37 isolates to piperacillin-tazobactam (69.8%), 33 isolates to ampicillin-sulbactam (62.2%), 32 isolates to ceftriaxone (60.3%). Also, Out of 49 isolates resistant to fluoroquinolones, 46 mutations were observed in *gyrA* gene and 25 mutation samples were observed in *parC* gene.

### CONCLUSION

Moreover, Although *parC* mutation is always necessary with mutation in *gyrA* to obtain high resistance to quinolones, three clinical samples in this study had mutations in *parC* without *gyrA*, so suggested that *parC* may not only be a secondary target for fluoroquinolones but maybe just as important as *gyrA* to increasing resistance to quinolones in *P. aeruginosa* isolates. Therefore, other mechanisms for antibiotic resistance of *P. aeruginosa* such as efflux systems and antimicrobial inactivating enzymes can be considered to clarify the mechanisms associated with *P. aeruginosa* resistance.

**Keywords:** *Pseudomonas Aeruginosa*, Fluoroquinolone-Resistant, Antibiotic Susceptibility

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antimicrobial effect of *Microbacterium oxidans* pigment against some Gram-positive and Gram-negative bacteria

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Some microorganisms produce pigments as secondary metabolites and exhibit differences in resistance to physical and chemical factors compared to non-pigmented strains. Microorganisms provide a readily us with sources of natural pigments. Fungal and bacterial strains have extensive advantages over plants due to pigment production, including rapid and easy growth in inexpensive environments, easy production process, and independence from weather conditions. Natural pigments derived from Actinobacteria have great potential as antimicrobial agents and can be beneficial in solving the problem of antibiotic resistance. *Microbacterium oxidans*, a member of the phylum Actinobacteria, has been found in many environmental habitats, including soil, plants, water, animals, food, and even rivers.

### MATERIALS AND METHODS

In this study, three culture media (ISP2 agar, nutrient agar, and starch casein agar) were investigated for growth and pigment production by *Microbacterium oxidans*. Then, the antibacterial activity of the *Microbacterium oxidans* supernatant at the best medium was investigated using the agar well diffusion method against pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Escherichia coli*. The pigment was extracted using water, methanol, and ethyl acetate solvents, and the obtained colored liquid was filtered, then the pigments were dissolved in dimethyl sulfoxide (DMSO) solvent. The antibacterial activity of the pigment of *Microbacterium oxidans* was then examined using the agar well diffusion method in Mueller-Hinton agar medium against the pathogens. Finally, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the pigment extract of *Microbacterium oxidans* were determined.

### RESULTS AND DISCUSSION

The ISP2 medium was the best culture medium for *Microbacterium oxidans* growth and pigment production among the three investigated media. The pigment extract showed good antimicrobial activity against *Staphylococcus aureus* (21 mm growth inhibition zone) and *Klebsiella pneumoniae* (32 mm growth inhibition zone). The pigment extract did not show any antimicrobial activity against *Escherichia coli* and *Bacillus cereus*. MIC and MBC were 35 $\mu$ l and 50 $\mu$ l for *Staphylococcus aureus* and 55 $\mu$ l and 70 $\mu$ l for *Klebsiella pneumoniae*.

### CONCLUSION

Bacterial pigments have a high potential as antimicrobial compounds, and *Microbacterium oxidans* is a suitable candidate for the industrial production of antibacterial pigments.

**Keywords:** Pigments, *Microbacterium oxidans*, Antimicrobial activity

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The application of Nanopore-Based Sensors in the Detection of Viral Infections

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Viral infections pose major threats to human health. The control of viral transmission as well as effective prevention of viral infections require accurate testing for the presence of viral particles. Conventional and standard methods for the detection of viruses, including polymerase chain reaction (PCR) assays, are often laborious and time-consuming. Sensors based on nanopores with adjustable size and low cost have been promising tools for the detection and characterization of viral particles owing to the fact that they enable real-time and direct virus identification instead of virus lysis and DNA or RNA extraction for sequencing.

### MATERIALS AND METHODS

Herein, we review recent advances regarding the application of nanopore-based sensors in the detection of viral particles by searching on "Google Scholar" database for publications.

### RESULTS AND DISCUSSION

A nanopore is a nanometer-scale hole which permits single molecule transportation through or in collision with the pore and the blockage event reveals the characteristics of the analyte, like charges, conformation, size, etc. Thus, the analyte can be identified. Various platforms of nanopore detecting, including biological nanopores, solid-state nanopores and hybrid nanopores which leverage the advantages of both types of nanopores, have been employed for the quantification of single viruses, characterization and quantification of antibody-virus interactions, identification of virus subtypes, monitoring of virus inactivation to assess inactivation methods, and precise characterization of individual viruses ; paving the way for point-of-care detection of viral infections.

### CONCLUSION

Nanopore-based sensors have evolved into powerful tools for real-time and efficient detection of viral particles as a result of dramatic advances in microfabrication and nanotechnology. Furthermore, due to being more portable whilst requiring less laboratory infrastructure, the utility of these sensors in clinical microbiology laboratories is highly plausible in the near future.

**Keywords:** Nanopores, Sensors, Viral infections, Virus detection

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation and identification of *Staphylococcus aureus* from meat products and detection of SEA producing gene in food samples related to poisoning

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Background and purpose: *Staphylococcus aureus* is known as one of the most common causes of food poisoning in many countries. This bacterium has many toxins, among which enterotoxin is more important, and consuming foods contaminated with this toxin causes food poisoning. Staphylococcal enterotoxins (SEs) are single-chain extracellular proteins with low molecular weight (22-29 kDa) that are similar in terms of biological activity but different in terms of antigens. These toxins have 23 different types that cause nausea, vomiting, diarrhea, muscle and abdominal pain, and sometimes lead to death. The purpose of this study is to review the role and importance of *Staphylococcus aureus* enterotoxins in food poisoning, pathogenesis, laboratory methods of purification and detection of these toxins.

### MATERIALS AND METHODS

By collecting more than 100 meat samples, the samples were examined for the presence of *Staphylococcus aureus* using standard culture techniques and standard phenotyping tests. After extracting DNA by authentic internal kits and designing specific primers and using the standard strain of *Staphylococcus* to identify the sea gene, a rapid PCR test with high specificity was performed. Based on the obtained results, *Staphylococcus aureus* was isolated in 19 samples (12.6%). The highest level of contamination was related to smoked fish (30%) and the lowest level of contamination was related to chicken schnitzel (3%), but no cases of *Staphylococcus aureus* contamination were observed in sausages. *Staphylococcus aureus* was counted using Bradparker agar medium. In the following, the frequency of genes of enterotoxins A-H and I, H, G and pseudo-J has been evaluated by PCR technique.

### RESULTS AND DISCUSSION

68% of the samples were infected with *Staphylococcus aureus*. The average number in raw and cooked minced meat samples, respectively, was equal to  $1.3 \times 10^5$  g/cfu and  $5.7 \times 10^3$  g/cfu, and among 92 isolates, 23 isolates, i.e. 25%, carried coding genes. They were enterotoxins. Of the above 23 isolates, 15 isolates (65.2%) carried one enterotoxin gene and the rest carried

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more than one gene. So that two isolates had SEA and SEC genes, two isolates carried SEA and SEE genes, one isolate carried SEA and SEG genes, one isolate carried SEC and SEI genes, one isolate carried SEC, SEA and SEG genes and one isolate carried SEE and SEG genes. Statistical investigations showed that there is a statistically significant difference in the comparison of the frequency of *Staphylococcus aureus* and the type of meat products. ( $p < 0.05$ ). Rosec and his colleague Gigaud in 2001 in France, a study with the aim of identifying Genes of classical staphylococcal enterotoxins Enterotoxins and (see and sea, seb, sec, sed) SEI, SEG, SEH and SEJ on 159 food samples Various, including cheese, milk, raw and cooked meat Pork, sweets, minced meat, ice cream and semolina. They made corn. 332 of all examined samples Staphylococcal strain isolation and according to the presence of genes Encoder of tested staphylococcal enterotoxins They decided They showed that 57% of the strains have Staphylococcal enterotoxin genes are often from The type of classic enterotoxins was staphylococci These are among the important research and the role of *Staphylococcus aureus* enterotoxins in Refers to health and public health.

### CONCLUSION

that the amount of food contamination with *Staphylococcus aureus* was relatively significant and due to the importance of *Staphylococcus aureus* in the production of various toxins and the most important cause of poisoning, due to the increasing consumption of ready-to-cook foods, it threatens the public health.

**Keywords: *Staphylococcus aureus* - enterotoxin SEA - PCR technique**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Structural characterization and functional annotation of hypothetical proteins in the drug-resistant *Pseudomonas aeruginosa* strains

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

*Pseudomonas aeruginosa* is one of the most dreaded bacteria in the world according to the World Health Organization (WHO) classification. This bacterium is one of the main causes of nosocomial infections with antibiotic resistance, severity and mortality enhancement. Thus, several attempts have been made nowadays to discover therapeutic targets. The present study aimed to find novel and common bacterial targets in drug-resistant strains of *Pseudomonas aeruginosa*.

### MATERIALS AND METHODS

The most drug-resistant and carbapenem-resistant strains of *Pseudomonas aeruginosa* were retrieved from databases and five common hypothetical proteins (HPs) with more than 200 amino acids were selected. The structural characterization and functional and features immunological annotation were predicted for these HPs with bioinformatics approaches.

### RESULTS AND DISCUSSION

A hypothetical protein (Gene ID: 2877781443) was revealed as the most possible drug and vaccine candidate among other investigated proteins based on structural and physicochemical, localization in cells, functional domains, peptide signals, virulence factor, toxicity, antigenicity and allergenicity predictions.

### CONCLUSION

The results of this study will contribute to design novel vaccine and drug candidates against *Pseudomonas aeruginosa* along with other HPs analysis and through experimental investigations.

**Keywords:** *Pseudomonas aeruginosa*, hypothetical proteins, drug resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### *Staphylococcus saprophyticus* carriage among healthy and pregnant women from southern Iran

Saba Karimi tabar<sup>a</sup>, Reza Khashei<sup>a,\*</sup>, Mozhddeh Momtahan<sup>a</sup>

#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Staphylococcus saprophyticus* as a genitourinary colonizer is an important agent of community-acquired urinary tract infections (UTI) in young females. We studied the microbiological and molecular characteristics of *S. saprophyticus* isolated from healthy women with sexual activity and pregnant women with UTI.

#### MATERIALS AND METHODS

Overall, 280 samples, consisting of 202 perineum swabs from healthy women and 78 urine specimens from pregnant women with UTI were included. Suspected *S. saprophyticus* from specimens were identified and confirmed using standard phenotypic and genotypic methods. Antimicrobial susceptibility against 10 drug was evaluated by using disk diffusion method. Six putative virulence genes were determined using PCR.

#### RESULTS AND DISCUSSION

A total of 31 (11.1%) *S. saprophyticus*, including 21 isolates from healthy women and 10 from pregnant women was obtained. The most effective antibiotics were found clindamycin and nitrofurantoin with 100% susceptibility. Of the 31 isolates, 100, 100, 100, 96.7, 90.3 and 74.2% were harbored *hrcA*, *ureC*, *dsdA*, *uafA*, *sssf*, and *ssp* genes, respectively. The *sdrI* gene was not detected in any of the isolates. A significant difference was shown between age and urinary catheterization with *S. saprophyticus* isolation.

#### CONCLUSION

The findings implicate the prevalence of relatively high of *S. saprophyticus* along with the presence of considerable virulence-associated genes and multiple drug resistance (41.9%) among them in our area. Monitoring of *S. saprophyticus* particularly in symptomatic pregnant women is necessary. To our knowledge, this is the first report on putative virulence genes of *S. saprophyticus* and the related risk factors for its carriage or infection status in Iran.

**Keywords:** *Staphylococcus saprophyticus*, healthy and pregnant women, PCR.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# ***Bordetella pertussis* CyaA toxin through regulating *RHO GTPases* pathway in human *can cause* whooping cough: In silico study**

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### **ABSTRACT**

#### **BACKGROUND AND OBJECTIVES**

*Bordetella pertussis* is an aerobic gram-negative coccobacillus and the reason of whooping cough. This bacterium causes disease exclusively in humans. It has shown bacterial proteins by interacting to human proteins cause many diseases. This interaction can change the signaling and metabolic pathways and cellular processes of infected host. Therefore, to identify molecular mechanism of *Bordetella pertussis* pathogenesis we decided to use bioinformatics software to find bacterial proteins that bind human proteins and then evaluated effected pathways.

#### **MATERIALS AND METHODS**

The analysis was done with bioinformatics software. To identify bacterial motifs that interact with human proteins, ImitateDB database was used. In this database, a strain of *Bordetella pertussis* was selected and motifs interacting with human cells were identified. Host interactor protein IDs were extracted from this database, and these codes were converted into gene symbols through BioDBnet database, and the function of these proteins was determined using string and EnrichR database.

#### **RESULTS AND DISCUSSION**

ImitateDB database showed Bifunctional adenylate cyclase toxin-hemolysin (CyaA) that plays a crucial role in host colonization can interact by CALM1 which is members of the EF-hand calcium-binding protein family. CALM1 also via different motif interact by 9 human proteins as figure 1 shown. As you can see from figure 2 Functional analysis by EnrichR indicated these genes significantly regulated RHO GTPases pathway. RHO GTPases pathway is key regulators involved in cell morphology, cell-matrix adhesion and cytoskeletal reorganization.

#### **CONCLUSION**

It is recommended to use more bacteria and bioinformatics software to identify different bacterial proteins that interact with human proteins and by examining the function of each protein, the way to predict and know more about the methods of causing disease by bacteria and prevention and let's treat diseases more smoothly.

**Keywords:** *Bordetella pertussis*, Bioinformatics analysis, ImitateDB database, BioDBnet database, string database, EnrichR database

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# **Helicobacter pylori cagA and vacA proteins through affecting on PI3K/AKT and Ras signaling pathways can induce gastric cancer: In silico study**

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### **ABSTRACT**

#### **BACKGROUND AND OBJECTIVES**

Helicobacter pylori infection is a critical risk factor for gastric cancer, contributing to approximately 75% of all gastric cancer (GC) cases. Therefore, today intensive research is focusing on molecular mechanisms in which H. pylori infection cause GC. It has shown bacterial proteins by interacting with human proteins cause many diseases. This interaction can change the signaling, metabolic pathways and cellular processes of infected host. Therefore, we decided to use bioinformatics tools to find how H. pylori can induce cancer in human gastric cells.

#### **MATERIALS AND METHODS**

To identify bacterial motifs that interact with human proteins, ImitateDB database was used. In this database, the H. pylori strain was selected and proteins that interact with human cells were recognized. Host interactor protein IDs were extracted from this database, and these codes were converted into gene symbols through BioDBnet database, and the function of these proteins was determined using string and EnrichR database.

#### **RESULTS AND DISCUSSION**

ImitateDB database showed H. pylori Cytotoxicity-associated immunodominant antigen (cagA) and Vacuolating cytotoxin A (vacA) can interact by human Tyrosine-protein phosphatase non-receptor type 11 (PTPN11) and NCK-interacting protein with SH3 domain (NCKIPSD) respectively. These two proteins also via different motifs relate to 55 human proteins as figure 1 shown. As you can see from figure 2 functional analysis by EnrichR indicated these genes significantly regulated PI5P, PP2A and IER3 Regulate PI3K/AKT and Ras signaling pathway. Different studies have shown RAS and PI3K/AKT signal transduction pathways dysfunctions have been involved in multiple cancer types including GC.

#### **CONCLUSION**

Today finding the interaction between host proteins and bacterial proteins through different databases and software can be used as an effective way to investigate molecular mechanisms of bacterial pathogenies and find appropriate treatments.

**Keywords:** Helicobacter pylori, Bioinformatics analysis, Ras signaling, ImitateDB database, BioDBnet database, EnrichR database

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence and molecular epidemiology of ceftaroline non-susceptible methicillin-resistant *Staphylococcus aureus* isolates, first clinical report from Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogens in Iran with a high prevalence and a high level of antibiotic resistance. Ceftaroline is a fifth generation cephalosporin binding and inhibiting penicillin binding protein (PBP2a).

### MATERIALS AND METHODS

In the present study, 228 clinical MRSA isolates were collected from four cities of Iran and their susceptibility to ceftaroline was evaluated by E-test and the disk diffusion method.

### RESULTS AND DISCUSSION

Our results showed a high susceptibility rate (97.3%) to ceftaroline in MRSA strains from Iran. Six isolates were found to be ceftaroline non-susceptible (CPT-NS) with Minimum inhibitory concentration (MIC)  $\geq 2$  mg/mL. All CPT-NS isolates were isolated from blood and tracheal aspirate and belonged to SCC $mec$  type III as well as *agr* type I and were all susceptible to vancomycin. Out of six isolates, three, two and one belonged to *spa* type t030, t4864, and t969, respectively. Vancomycin, quinupristin/dalfopristin, linezolid, chloramphenicol, and tigecycline were the most active agents against CPT-NS isolates.

### CONCLUSION

Due to the broad-spectrum activity and low toxicity of ceftaroline as well as the increased rate of vancomycin resistance among MRSA strains in recent years, ceftaroline can be considered as a novel approach to treat MRSA-induced infections.

**Keywords:** ceftaroline, Fifth-generation cephalosporin, MRSA, *Staphylococcus aureus*, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Synthesis and characterization of magnesium nanoparticles loaded with lactoferrin and evaluation of its antibacterial properties

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nanoparticles enhance infectious illness diagnosis and therapy. Antibacterial nanoparticles are intriguing because of their tiny sizes, high surface-to-mass ratios, and other peculiar physical and chemical properties. Metal oxide-based nanoparticles may swiftly infiltrate bacterial cell walls, increasing ROS generation and blocking cell wall enzymes. Lactoferrin (LF) has antibacterial properties as it is preventing bacteria from obtaining a critical food supply. Therefore, LF has ability to kill germs. In this study, we aimed to synthesis and characterize magnesium nanoparticles (MgNPs) loaded with LF and evaluate their antibacterial properties.

### MATERIALS AND METHODS

The sol-gel technique was used to manufacture the MgNPs. Surface modification was then used to load LF. Physical characteristic of Mg-LF such as particle size, zeta potential and homogeneity were analyzed by zetasizer. Standard agar diffusion and minimum inhibitory concentration (MIC) tests were used to find out how well LF-loaded Mg NPs worked against Gram-negative bacteria like *Pseudomonas aeruginosa*.

### RESULTS AND DISCUSSION

Our results demonstrated the successful synthesis of LF-loaded MgNPs with an average size of 18-20nm. Mg nanoparticles have a negative charge and LF is trapped by Mg nanoparticles because it has a positive charge. The LF-loaded MgNPs exhibited no significant antibacterial activity against *Pseudomonas aeruginosa*, as evidenced observed in the agar diffusion assay. MIC values revealed no effective bactericidal activity in *Pseudomonas aeruginosa* strains.

### CONCLUSION

In conclusion, LF-loaded magnesium nanoparticles were created. The Bradford test and Zetasizer results were showed that and their properties were analyzed Mg NPs were efficiently entrapped LF by electrostatic interactions and inhibited that from protease degradation. But against other researches LF-loaded MgNPs was not had any antibacterial activity, because MgNPs were changed LF structure that was confirmed by UV-Visible and Fluorescence spectroscopy.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Association between maternal food group intake during pregnancy and maternal and infant gut microbiota

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The maternal diet during pregnancy provides all nutrient requirements to optimize the fetus's growth. However, it can also affect the infant's health by modulating its gut microbiota composition in utero. Forming the first neonatal microbiota composition may play an essential role in determining the susceptibility to various diseases in the future. Since a healthy intrapartum dietary pattern can affect both mother and infant microbiota and their long-term health, we aimed to investigate the association between maternal food group intakes during pregnancy and maternal and infant gut microbiota.

#### MATERIALS AND METHODS

In this prospective cohort study, 46 mothers were included in the third trimester of pregnancy. Overall 5 24-hour dietary recalls were recorded for each of them. Mother's stool and infant meconium samples were collected after birth in an aseptic condition. DNA was extracted and PCR, and maternal-neonatal microbiota profiling were assessed by universal-16SrRNA gene sequencing.

#### RESULTS AND DISCUSSION

Out of 724 bacteria found in 50 genera in the mothers' stool samples and 272 in 25 genera in the infants' meconium samples, the highest frequency of genera and number was gracilicutes bacteria in both the mother and infant. 16 bacteria were common in mother and neonate flora. 34 and 6 bacteria were specific for the mother and neonate intestinal microbial population, respectively.

#### CONCLUSION

Our findings demonstrated that dietary intake during pregnancy is associated with maternal and neonatal gut microbiota.

**Keywords:** Diet, Gut microbiota, Pregnancy

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the effect of *Bifidobacterium bifidum* supernatant on mrkD gene expression of *Klebsiella pneumoniae* resistant to carbapenem and producing biofilm

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Bacterial resistance to antibiotics is one of the most important threats to human health. *Klebsiella pneumoniae* is one of these highly resistant bacteria that increase pathogenicity and higher resistance to antibiotics and host defense by forming a biofilm, and on this basis, the use of probiotics has been considered. We aim to determine the anti-biofilm and antibacterial effects of the supernatant isolated from *Bifidobacterium bifidum* on carbapenem-resistant *Klebsiella pneumoniae* and to determine the effects of the supernatant isolated from *Bifidobacterium bifidum* in reducing the expression of genes involved in the colonization and biofilm formation of *Klebsiella pneumoniae* resistant to carbapenems.

### MATERIALS AND METHODS

30 isolates of *Klebsiella pneumoniae* available in the archive of the laboratory were studied. A 24h culture was prepared from the stocks, and *Klebsiella pneumoniae* strains were confirmed with IMViC tests, and carbapenemase-producing strains were determined with mCIM, eCIM, and MHT tests. PCR was used to check the presence of the mrkD gene; Supernatant was prepared from *Bifidobacterium bifidum* and biofilm, antibiofilm, and MIC tests were performed, and finally Real-time PCR

### RESULTS AND DISCUSSION

We found that the effect of the supernatant of a probiotic (*Bifidobacterium bifidum*) on about 70% of *Klebsiella pneumoniae* strains with the mrkD gene and resistance to carbapenem decreases the expression of the mrkD gene.

### CONCLUSION

In this study, we found that the use of probiotics can partially help to combat antibiotic resistance in carbapenem-resistant *Klebsiella pneumoniae* bacteria.

**Keywords:** *Klebsiella Pneumoniae*, *Bifidobacterium Bifidum*, Mrkd , Carbapenemase , Probiotics

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Effectiveness of Mycobacterium tuberculosis recombinant proteins-coated gold nanoparticles in improving the interferon gamma release assay test

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

In the last few decades, it has become important to find new assays to diagnose tuberculosis. Gold nanoparticles (GNPs) are chemically inert, have low toxicity, and are easy to modify and functionalize for the detection of many pathogens. They have excellent immune modulatory and adjuvant properties. In this study, GNPs were coated with recombinant *Mycobacterium tuberculosis* proteins, including TB10.4 (Rv0288), CFP-10 (Rv3875), ESAT-6 (Rv3874) and TB7.7 (Rv2654c) and exposed to the whole blood of subjects with active tuberculosis (aTB), Latent tuberculosis infection (LTBI) and healthy controls (HC). Then, the level of interferon gamma (IFN- $\gamma$ ) produced in the serum of the subjects was measured and compared with the level of IFN- $\gamma$  produced in tubes without GNPs.

### MATERIALS AND METHODS

The TB 7.7 gene was extracted and cloned from *Mycobacterium tuberculosis* H37Rv. This antigen was expressed and purified along with other antigens: CFP-10, ESAT-6, and TB 10.4. Also, to increase the stability of recombinant proteins, different formulas prepared from additive compounds were investigated. Gold nanoparticles coated with the optimal concentration of proteins. Whole blood samples from subjects were collected in tubes containing QFT-A (CFP-10, ESAT-6, TB7.7, TB10.4), QFT-B (CFP-10, ESAT-6, TB7.7), QFT-NG (proteins coated with gold nanoparticles), TB1 and TB2 tubes (QFT-G-IT). Secreted interferon gamma (IFN- $\gamma$ ) was measured by ELISA method and results were analyzed by statistical methods.

### RESULTS AND DISCUSSION

The results showed that the IFN- $\gamma$  production in the GNPs tubes (QFT-NG) was significantly higher than in tubes without GNPs [QFT-A: (CFP-10, ESAT-6, TB7.7, TB10.4), QFT-B: (CFP-10, ESAT-6, TB7.7)] in aTB, LTBI, and HC subjects. The QFT-NG tube with the QFT-A tube (aTB): mean difference (MD = 0.44, 95 % confidence interval: 0.07-0.88) and the QFT-NG tube with the QFT-A tube (LTBI): (MD = 0.21, 95% CI: 0.15-0.4). Further, the group's analysis revealed that the MD of IFN- $\gamma$  production between the QFT-NG, QFT-A, and QFT-B tubes was significantly higher in aTB subjects than in LTBI subjects. Interestingly, IFN- $\gamma$  production was lower in aTB and LTBI subjects in each of the TB1 and TB2 tubes than in the QFT-NG tube.

### CONCLUSION

Interferon-gamma release assays with improved sensitivity by adding gold nanoparticles increase the possibility of more accurate and faster detection of active TB and LTBI subjects.

**Keywords:** *Mycobacterium Tuberculosis*, Gold Nanoparticle, Active Tuberculosis, Latent Tuberculosis Infection, Interferon Gamma, Recombinant Proteins

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The role of *Staphylococcus aureus* enterotoxin B in chronic rhinosinusitis with nasal polyposis

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### ABSTRACT

CRS with nasal polyps (CRSwNP) is a multifactorial disease, and various etiological factors like bacterial superantigens are known to develop this disease. Recent studies reported that *Staphylococcus aureus* nasal colonization was detected in 67% of the patients with CRSwNP. Moreover, it was reported that specific IgE against *S. aureus* enterotoxins are discovered in almost half of the nasal tissue homogenates from nasal polyps. Thus, investigations have highlighted the role of staphylococcal enterotoxins, especially enterotoxin B (SEB), in pathogenesis of CRSwNP.

The destruction of mucosal integrity was reported as a main SEB-related pathogenic mechanisms in CRSwNP. SEB activates Toll Like Receptor 2 and triggers the production of pro-inflammatory cytokines; furthermore, it induces reactive oxygen species and endoplasmic reticulum stress-induced inflammation that may cause epithelial cell integrity disruption and enhance their permeability. SEB-induced Type 2/Th2 pathway results in degranulation of eosinophils, cationic proteins production, and localized eosinophilic inflammation. Furthermore, SEB may be involved in the expression of RORC and HIF-1 $\alpha$  in Tregs and by maintaining the inflammation in sinonasal mucosa that could have a main role in the pathogenesis of nasal polyposis. Different in vitro findings were confirmed in animal studies; however, in vivo analysis of SEB-induced nasal polyps and CRS remains unfulfilled due to the lack of appropriate animal models. Finally, after elucidating different aspects of SEB pathogenesis in CRSwNP, therapeutic agents have been tested in recent studies with some encouraging results.

### CONCLUSION

The purpose of this article is to summarize the most important findings regarding SEB-induced CRS and nasal polyposis.

**Keywords:** Chronic rhinosinusitis, Type 2/Th2 pathway, *Staphylococcus aureus*, Enterotoxin B, Nasal polyps

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### A systematic review of case reports of hepatic actinomycosis

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Hepatic Actinomycosis (HA) is one of the infections that causes disorders in patients when diagnosed untimely and inappropriately.

#### MATERIALS AND METHODS

Case reports on HA in patients published between 2000 and April 2020 were gathered by carrying out a structured search through PubMed/Medline.

#### RESULTS AND DISCUSSION

Through a survey of the Medline database, 130 studies were identified and then, 64 cases with HA were included in the final analysis. Asia had the largest share of cases with 37.5% (24 reports), followed by Europe and the Americas. Affected patients were predominantly males (64%) and the overall mortality rate was 1% with only one male patient in his 50 s dying. Nearly all patients (92%) were immunocompetent. However, in four patients, the use of immunosuppressive medication led to depression of the immune system. Most of the patients (80%) experienced complications. In terms of the complications, the most frequent ones were previous history of abdominal surgery (32%) and foreign bodies in the abdominopelvic region (20%). *Actinomyces israelii* was the most common pathogen isolated from patients. Abdominal pain (66%), fever (62%), weight loss (48%), night sweat, malaise, and anorexia (14%) over about 3.1 months were the most frequently reported clinical symptoms. Extension to one or more surrounding organs was evident in 18 patients (28%). Histopathologic examination confirmed infection in 67% of the patients and samples obtained from liver puncture biopsy (32%) were most frequently used in diagnosis. Surgery or puncture drainage+anti-infection was the most common method to treat patients and penicillin, Amoxicillin, Doxycycline, and ampicillin were the most frequently used drugs to control infection.

#### CONCLUSION

HA should be considered in patients with a subacute or chronic inflammatory process of the liver. With accurate and timely diagnosis of infection, extensive surgery can be prevented.

**Keywords:** Actinomycosis, Liver abscess, Hepatic actinomycosis, *Actinomyces* species, Diagnosis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Immunoprotectivity of an antigenic construct derived from Omp34 against *A. baumannii* in murine pneumonia model

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

*Acinetobacter baumannii* has emerged as one of major nosocomial pathogen that can cause various hospital infections, including pneumonia, sepsis, meningitis, post-traumatic infections and urinary tract infections. Its infections due to global emergence of multidrug-resistant even colistin strains has become a challenge for developing effective treatment options that putting the world on the brink of a post-antibiotic era. This problem leads researchers to the development of prophylactic vaccination to prevent this pathogen infections. Nasal vaccination could provide a strong mucosal and systemic immunity to combat these infections. Therefore, it is considered a promising strategy for the prevention of diseases, especially infectious diseases of the respiratory system. The development of nasal vaccines, particularly the strategies of adjuvant and antigens design and optimization enabling rapid induction of protective mucosal and systemic responses against the disease. In this context, chitosan is recognized as an established mucosal adjuvant/delivery system that has been extensively studied owing to its low toxicity, adhesion, pro-permeability, immunostimulant, ability to be absorbed in human tissues, and excellent histocompatibility with human tissues and organs. Therefore, multivalent vaccines containing different antigens are suitable candidates for providing admissible protection, and this study is focused on an outer membrane protein, Omp34 and a structure derived from it, L3X5 as a multivalent vaccine.

### MATERIALS AND METHODS

Immunogenic peptide Omp34L3X5 was transferred to the *E. coli* BL21 expression host and with recombinant protein Omp34 were expressed, purified, and nasally administrated into BALB/c mice individually and in combination with chitosan. Active immunization was carried out. The mice were then challenged with a clinical isolate of *A. baumannii*. Then, the level of antibody in mice was measured by Indirect ELISA. The protective effect of the antibody produced against the mentioned antigens was evaluated by determining the load of bacteria in the lungs, spleen, and liver organs, as well as the animal's survival.

### RESULTS AND DISCUSSION

ELISA analysis revealed increased antibody production in all immunized groups. However, the combined administration of the proteins with chitosan provided superior protection compared to administering each antigen individually.

### CONCLUSION

Polyvalent vaccines containing several antigens provide acceptable protection with the use of appropriate adjuvants.

**Keywords:** *Acinetobacter baumannii*., Omp34., L3X5., Chitosan., Vaccine., Immunogenicity

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of biofilm formation and antibiotic resistance patterns in *Staphylococcus epidermidis* isolated from food samples in Shahroud, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Staphylococcus epidermidis*, a member of Coagulase-negative staphylococci group, can contaminate a variety of food products because humans are common carriers of this bacterium and may be related to specific human infections. This study aimed to evaluate phenotypic detection of the biofilm formation was done by the Congo Red Agar (CRA) method and antimicrobial resistant of *Staphylococcus epidermidis* strains isolated from food samples in shahroud, Iran.

### MATERIALS AND METHODS

In present study, 100 food isolates of *S. epidermidis* were identified by using conventional microbiological and biochemical standard tests and then were tested for biofilm formation on Congo red agar (CRA) and the antimicrobial resistance testing of each isolate was examined using the disk diffusion method (Kirby-Bauer technique), on Müeller-Hinton agar plates. The following antibiotic disks used were ciprofloxacin (5µg), SXT (5µg), erythromycin (25µg), gentamycin (10µg), Cefoxitin (30µg), clindamycin (2µg), rifampin (5µg), tetracycline (30µg), Chloramphenicol (30µg) (MAST, Berkshire, U.K.).

### RESULTS AND DISCUSSION

In recent decades, *S. epidermidis* infections are an increasing cause of concern, because of distribution and high spread of antibiotic resistance among the isolates and their persistence on biotic and abiotic surfaces. The ability to attach to indwelling medical devices and food-processing surfaces and subsequently biofilm production is one of the most prevalent virulence factors in *Staphylococcus epidermidis*- associated infections. This microorganism is more frequent in dairy and meat products and the most predominant causative agents of hospital associated infections. A total of 134 food specimens including raw meat (n = 30) and raw chicken (n = 23), creamery cake (n=35), dried whey (n=12), dough (n=12) cheeses (n=5), and cream (n= 17) were collected. Among 100 *S. epidermidis* isolates examined only 19 (19%) food isolates produced black colonies (biofilm-producing strains) on CRA plates. All *S. epidermidis* isolated from food samples showed no resistance to linezolid, ciprofloxacin, and gentamicin. Twenty six (26%) revealed resistance to erythromycin, 16 (16%) to clindamycin, 11 (11%) to tetracycline, 9 (9%) to SXT, 7 (7%) to rifampin. and 2 (2%) to chloramphenicol.

### CONCLUSION

Isolation of *Staphylococcus epidermidis* isolates with ability to form biofilm and antibiotic resistance from food samples is a warning for us and requires more detailed molecular investigations.

**Keywords:** *Staphylococcus epidermidis*, CRA, Food samples, Shahroud

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Enhancing Cell Viability and Immunogenicity of OmpA as a Promising Subunit Vaccine Candidate Against Cytotoxic Effects of *A. baumannii*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Acinetobacter baumannii* is a significant opportunistic pathogen and a major concern in healthcare settings due to its involvement in nosocomial infections and high antimicrobial resistance. The alarming mortality rate among infected patients underscores its success as a pathogen. Outer membrane protein A (OmpA) has been identified as a crucial virulence factor associated with the survival and pathogenicity of *A. baumannii*. OmpA interacts with eukaryotic cells, leading to cytotoxicity by binding to death receptors on the cell surface. Additionally, OmpA plays a significant role in bacterial pathogenesis by interacting with epithelial cells, inducing apoptosis, and inhibiting complement activation.

### MATERIALS AND METHODS

The OmpA was expressed and purified. The purified protein was then injected into groups of mice to induce the production of anti-OmpA antibodies. HeLa cells were plated at 70% confluency. The standard strain of *A. baumannii* ATCC 19606 and a clinical isolate, *A. baumannii* 58ST, were exposed to Anti-OmpA serum. The cells were incubated with the bacteria-cell solution overnight. MTT test was performed.

### RESULTS AND DISCUSSION

OmpA was successfully expressed, purified, and visualized as ~ 38kDa on SDS-PAGE. An increased antibody titer was achieved as measured by indirect ELISA. Cells exposed to the sera showed lower infections than the control group.

### CONCLUSION

The presence of anti-OmpA antibodies enhances cell viability against the cytotoxic effects of *A. baumannii*. OmpA demonstrates immunogenicity and holds promise as a candidate for the development of an effective subunit vaccine against *A. baumannii* infections.

**Keywords:** *Acinetobacter baumannii*, OmpA, Cytotoxic effects, HeLa cell line, Subunit vaccine

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Inhibition of biofilm formation by serum raised to the outer membrane protein A of *Acinetobacter baumannii*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Biofilm production is one of the various virulence factors of *Acinetobacter baumannii* that helps the organism resist stressful conditions and facilitates the development of intense infections and diverse resistance mechanisms. Biofilms allow *A. baumannii* to survive and spread in hospital environments by attaching to different surfaces, such as cerebrospinal fluid shunts, and vascular catheters. Outer membrane protein A (OmpA) is a prominent porin in the outer membrane of *A. baumannii*. This protein plays a crucial role in bacterial pathogenesis by contributing to biofilm formation, interaction with epithelial cells, induction of apoptosis, and inhibition of complement, and serves as a potential vaccine candidate against *A. baumannii* infections.

### MATERIALS AND METHODS

We expressed recombinant protein OmpA (rOmpA). Purification was achieved using imidazole gradient of Ni-NTA column chromatography and confirmed with SDS-PAGE. The protein concentration was determined via Bradford colorimetric method. The purified OmpA was injected to BALB/c mice, and serum antibody titer was assessed using indirect ELISA. *A. baumannii* strains were exposed to OmpA serum, resuspended, and incubated in 96-well plates. Crystal violet staining and ELISA reader absorbance measurements followed after washing and decolorization with acetic acid.

### RESULTS

The protein expression and purification were confirmed by analyzing the SDS-PAGE gel, which showed the protein at the expected position (approximately 38 kDa) and ensured correct protein folding. The indirect ELISA results indicated an increased antibody titer in immunized mice compared to the control group. When the serum was challenged with the bacteria, it was observed that the serum reduced biofilm production in both the standard and clinical strains of *A. baumannii*.

### CONCLUSION

The recombinant OmpA protein induced a protective effect against *A. baumannii* in mice and prevented biofilm formation in strains of *A. baumannii*. OmpA is considered as a promising subunit vaccine candidate against *A. baumannii* infections.

**Keywords:** *Acinetobacter baumannii*, OmpA, Biofilm formation, Subunit vaccine

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### A review of the antibacterial and antiparasitic effects of *Matricaria chamomilla* L. on infectious diseases

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#### ABSTRACT

Data about *M. chamomilla* were gathered using scientific search engines including Web of Science, PubMed, Wiley Online, SpringerLink, ScienceDirect, Scopus, and Google Scholar.

Herbal remedies offer accessibility, cultural factors, individual preferences, natural and organic products, and synergistic effects for centuries-old treatment

*M. chamomilla*, more often known as chamomile, is a well-known medicinal plant that belongs to the Asteraceae family. It is an annual plant that thrives in a variety of soil types and can withstand cold conditions. It is widely used in conventional medicine to treat a wide range of diseases, including infections

It is used as an infusion against several infectious diseases, such as female genital infections, infections of the mouth, and eye infections. Externally, the drug in powder form may be applied to wounds slow to heal, skin eruptions, and infections, such as shingles and boils, as well as haemorrhoids.

The delicatest strain was *Staphylococcus aureus*, while the most resistant one was *Pseudomonas aeruginosa*. The strain that was most vulnerable was *Staphylococcus aureus*, whereas the strain that was most resistant was *Pseudomonas aeruginosa*. Gram-negative bacteria may be more vulnerable to *M. chamomilla* essential oil (EO) than Gram-positive ones are.

methanol and various *M. chamomilla* extracts, such as the alcoholic and ethanolic extracts. Aqueous extracts have demonstrated antibacterial effects against diverse bacterial strains, while extracts and essential oils have demonstrated antibacterial characteristics.

The antibacterial effects induced by *M. chamomilla* extracts and essential oils are likely a result of multiple mechanisms, including membrane disruption, inhibition of essential enzymes, and interference with bacterial adhesion and biofilm formation.

Essential oils and extracts from *M. chamomilla* have been demonstrated in studies to suppress the development of insects and parasites. *Leishmania amazonensis* and *Leishmania infantum* exhibited good activity in in vitro tests, and bisabolol activated programmed cell death effects. The combination of *M. chamomilla* and *Foeniculum vulgare* hexane extracts had larvicidal efficacy against *Culex pipiens*, while the methanolic extract showed significant anti-acanthamoeba activity. Therefore, the use of the mentioned plant is due to its antibacterial and anti-parasitic properties, it also has antioxidant, antifungal, and anti-inflammatory properties, which has highlighted the plant as a suitable candidate either individually or in combination with other drugs.

**Keywords: Antibacterial and Antiparasitic Effects, *Matricaria Chamomilla* L.**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluate the effect of *Protopermaliopsis muralis* on *icaA* gene expression in *Methicillin-Resistant Staphylococcus aureus* isolated from burn wound

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Nowadays, the emergence and alarming rise of multidrug resistant pathogenic bacteria is becoming a serious health challenge. So, there is an urgent need to obtain novel ingredients with antimicrobial activity. In this regard, lichens are promising substance with antimicrobial potential to control different microbial strains such as fungi, gram-positive/-negative bacteria and viruses. In this study we aimed to determine the use of *Protopermaliopsis muralis* in the treatment of burn wound infection.

#### MATERIALS AND METHODS

Twenty-four *Methicillin-Resistant Staphylococcus aureus* isolates were recovered from burn wound infections of patients admitted to the burn ward of Besat hospital of Hamadan, Iran, during 2022. The minimum inhibitory concentration (MIC) of *Protopermaliopsis muralis* was determined by microdilution broth method. The effect of MIC and sub-MIC concentrations of this extract on biofilm formation was determined by the microtiter plate method (MTP). Also, the expression level of the *icaA* gene was assessed by Real-Time PCR technique.

#### RESULTS AND DISCUSSION

According to the results, the extract from *Protopermaliopsis muralis* demonstrated MIC and MBC at a concentration of 625 µg/ml and 1250 µg/ml for *MRSA* isolates. Additionally, this extract demonstrated 73% biofilm formation inhibition. Also, bacterial cells exposed to *Protopermaliopsis muralis* showed 1fold down-regulations of *icaA* gene expression levels.

#### CONCLUSION

This study showed the important role of *Protopermaliopsis muralis* in enhancing antibacterial and antibiofilm activity which can be used in the prevention of *biofilm* established by *MRSA* in vitro models.

**Keywords:** *Protopermaliopsis muralis*, Biofilm, *Methicillin-Resistant Staphylococcus aureus*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Studying the survival of epithelial cells in the presence of native probiotic strains

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Probiotics have attracted a lot of attention due to their potential therapeutic applications and the production of various metabolites such as vitamins. In this study, the survival of epithelial cells in the presence of 7 probiotic strains and the production of some B vitamins were investigated.

### MATERIALS AND METHODS

In order to measure the viability, A549 cells were added to the number of  $10^4 \times 4.5$  cells in each well of the 96-well plate. Next, the cell sediment of probiotic bacteria was washed thrice with sterile phosphate buffered saline and added to the culture medium containing metabolites of probiotics with cell dilution series of  $10^8$ ,  $10^6$  and  $10^5$  cfu/ml. After 24 hours of incubating, the survival of epithelial cells were studied by MTT assay. To measure vitamin production by probiotic strains, 20  $\mu$ l of culture medium containing metabolites of probiotics and solution of vitamins B2, B3 and B6 were coated on chromatography paper and immersed in the mobile phase for separation. Chromatograms were developed and, after drying, viewed under a UV lamp

### RESULTS AND DISCUSSION

The results showed that eukaryotic cells have a potential to survive in the presence of  $10^5$  dilution of probiotics in the range of 40 to 100%, and survival is directly correlated with the quantity of bacteria. *SUBC4* and *SUBC2* strains had the lowest and highest survival rates in dilution  $10^8$ , with 75% and 100%, respectively. As Song et al. studied effect of probiotics on gastric epithelial cells in 2019. Additionally, in TLC, the results of calculating the  $R_f$  value, revealed that probiotic strains produce the vitamins B2, B3, and B6. As Indira, M., et al. studied bioactive molecules produced by probiotics at 2019.

### CONCLUSION

A promising strategy for advancing both health and innovation in the food industry is the inclusion of probiotics in food products.

**Keywords:** Cell Viability, MTT, Probiotics, Thin layer chromatography, Vitamin B.

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# Protectivity of a recombinant protein derived from a 5×Loop3 of Omp34 against *Acinetobacter baumannii* in a murine sepsis model

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Acinetobacter baumannii* is a globally recognized primary cause of infections acquired in hospitals. This bacterium, although considered a low-grade opportunistic pathogen, plays a crucial role in causing various types of infections such as ventilator-associated pneumonia, urinary tract infection, skin and wound infections, bacteremia, and meningitis. In light of the emergence of antibiotic-resistant strains, alternative treatment strategies like recombinant vaccines and specific antibodies have gained importance for combating these infectious bacteria. However, the effectiveness of tested bacterial surface antigens has been limited to providing partial protection. Hence, the development of polyvalent (multi-antigen) vaccines incorporating different antigens has become imperative to achieve a satisfactory level of protection. In this study, the focus is on utilizing two outer membrane proteins, namely construct L3x5 and Omp34, as components of a polyvalent vaccine

### MATERIALS AND METHODS

It includes plasmid purification, expression, purification, and injection of construct L3x5 and Omp34 recombinant proteins into BALB/c mice. Both active and passive immunizations were performed and organ transplantation. The mice were subsequently challenged with a clinical isolate of *A. baumannii*. The antibody levels in mice were measured using Indirect ELISA. The survival rate of the challenged animals was also determined

### RESULTS AND DISCUSSION

ELISA analysis revealed increased antibody production in all immunized groups. However, the combined administration of the L3x5 construct and Omp34 proteins provided superior protection compared to administering each protein individually. The protein OMP34 is an inclusion body and the structural protein L3X5 is soluble.

### CONCLUSION

Polyvalent vaccines containing multiple antigens offer a satisfactory level of protection.

**Keywords:** *Acinetobacter baumannii*; Recombinant protein; construct L3x5, Omp34; Immunogenicity; Vaccine

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Screening of effective bacteriophages against *Pseudomonas aeruginosa* from Dasht Desert

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Antibiotic-resistant *Pseudomonas aeruginosa* is the common causes of hospital infections, Therefore, bacteriophage therapy is a suitable alternative for the treatment of *P. aeruginosa* infections. This study aimed to isolate of effective bacteriophages against *P. aeruginosa*

### MATERIALS AND METHODS

In this study, soil samples were collected from a garden located in Semnan province. Soil and sterile distilled water were mixed in a 1:1 (w/v) ratio and shaken for 8 hours at 30°C. Then this mixture was centrifuged and supernatant was filtered. The filtered supernatant was mixed with equal amount of nutrient broth, 25 µl of *Pseudomonas aeruginosa* culture and shaken at 37°C for 36 hours. After centrifugation and filtration, phage lysate was concentrated using NaCl and polyethylene glycol 8000. Plaques morphology and number of phages in concentrated solution, MOI value and stability of phage in different physical conditions were studied by double layer agar method. Transmission electron microscopy (TEM) was used to determine the morphology and phage family.

### RESULTS AND DISCUSSION

Phage plaques produced spherical shape with a semi-clear halo around the center. The number of bacteriophage in the phage concentrate was equivalent to  $3.5 \times 10^{11}$  PFU/mL, the MOI value is 8.75 and most stable at 4°C and pH 7. The morphology analysis with TEM, showed that phage had an icosahedral head with a contractile tail about 140 nm that belongs to the myoviridae family. In 2021, Lashtoo Aghaei et al isolated eighteen different bacteriophage against *Pseudomonas aeruginosa* from sewage and showed that bacteriophage were most effective in lysing *Pseudomonas aeruginosa*. In 2022, Abo Kamer et al isolated and identified a new bacteriophage against *Pseudomonas aeruginosa* and evaluated its potential effect in the treatment of skin infection.

### CONCLUSION

The results of these preliminary investigations indicate the bacteriophages may be considered for use in phage therapy against *Pseudomonas aeruginosa*.

**Keywords:** Myoviridae, Dasht Desert. Phage Therapy, *Pseudomonas aeruginosa*.

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# Protective effects of probiotic mixture (*Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus heloticus*) on Doxorubicin--induced Hepatotoxicity in Rat Liver

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Doxorubicin (DOX), a common antibiotic used to treat a variety of tumors, but its side effects limit its clinical use. Therefore, finding effective protective agents to combat DOX-induced organ damage is a necessity. The purpose of this study is to determine the protective role of probiotic mixture (*Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus heloticus*) on Doxorubicin-induced Hepatotoxicity in Rat Liver

### MATERIALS AND METHODS

In this experimental study, 21 male Wistar rats were divided into three groups: control, recipient of DOX (20 mg/kg), recipient of DOX+ and recipient of probiotic mixture (10<sup>9</sup> CFU/ml). Hepatotoxicity was induced as a single dose by intraperitoneal injection of DOX and probiotic mixture by gavage method for 30 days. After the treatment period, in order to measure serum liver damage markers (ALT, AST, total and direct bilirubin), NO and MDA levels, blood was taken from the heart area of the animals. Data analysis was done in different groups with SPSS software and one-way variance statistical test. and P<0.05 was considered significant.

### RESULTS AND DISCUSSION

: The results of the study showed that the level of liver serum markers (ALT, AST, total and direct bilirubin), NO and MDA in the group receiving DOX showed a significant increase compared to the control (P<0.001). In the group receiving the probiotic mixture, serum levels of ALT, AST, total and direct bilirubin, NO and MDA levels were significantly reduced compared to the group receiving with DOX (P<0.05).

### CONCLUSION

The results suggest that liver protection caused by the consumption of probiotic mixture in rats receiving DOX is probably related to the inhibition of oxidative and antioxidant stress.

**Keywords:** Probiotic, Doxorubicin, liver serum markers, NO, MDA, Rat

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# Protective effects of probiotic mixture (*Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus heloticus*) on carbon tetrachloride-induced damage in kidney tissue of male Wistar rats

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Carbon tetrachloride (CCl<sub>4</sub>) by producing free radicals causes damage in various tissues, including the kidney. In this study, the protective effect of probiotic mixture (*Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus heloticus*) on tetrachloride-induced damage on kidney tissue of male rats was investigated.

### MATERIALS AND METHODS

In this experimental study, 21 male rats (weight range 200-220 g) were randomly divided into 3 groups of 7 including: control group, carbon tetrachloride group (2 mg/kg) and receiving carbon tetrachloride + probiotic mixture (*Lactobacillus casei*, *Lactobacillus Rhamnosus* and *Lactobacillus heloticus*) (CFU/MI10<sup>9</sup>). CCl<sub>4</sub> was by intraperitoneal injection and receiving probiotics for 35 days by gavage method. After the end of the treatment period; to measure serum levels of creatinine, total protein and albumin, blood was taken from the heart area. Then, the kidneys were removed from the animals for histological examination. Data analysis in different groups was done with SPSS software and one-way variance statistical test, and P<0.05 was considered significant.

### RESULTS AND DISCUSSION

CCl<sub>4</sub> injection to rats significantly increased the serum creatinine level compared to the control group (P<0.001), while it decreased the serum albumin and total protein levels. Also, treatment with probiotic mixture significantly caused significant changes in the receiving group compared to the carbon tetrachloride receiving group (P<0.01). Treatment with probiotic mixture caused improvement in the histopathological changes of kidney tissue sections.

### CONCLUSION

The results of this study showed that the probiotic mixture (*Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus heloticus*) can moderate the toxic effects caused by carbon tetrachloride in the kidney, which is probably due to the antioxidant function of lactic probiotics.

**Keywords:** Probiotic, Carbon tetrachloride, Serum urinary factors, Kidney, Rat

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### Is there any difference between characteristics of nosocomial and community-acquired uropathogenic *E. coli* isolates?

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Nosocomial and community-acquired uropathogenic *Escherichia coli* (UPECs), the most common causative pathogen of urinary tract infection (UTI), have different phenotypic patterns which lead to the diversity in their pathogenicity, including the antibiotic resistance, influenced by differences in phylogenetic and biofilm formation ability. This study was conducted to evaluate the relationship between antibiotic resistance pattern and these characteristics in UPECs recovered from in-/out-patients with UTI in two hospitals of Tehran.

#### Materials and methods

From December 2022 to March 2023, bacterial samples were collected from the urine of UTI patients who referred to/hospitalized in Imam and Loghman hospitals. Of these, *E. coli* bacteria were confirmed using biochemical methods and PCR. Phylogenetic grouping was investigated using multiplex-PCR, and antibiotic sensitivity was determined using disc diffusion assay. Biofilm formation in the isolates was assessed using the microtiter assay.

#### RESULTS AND DISCUSSION

Sixty *E. coli* isolates (30 from each hospital) were recovered. Of these, 30 were isolated from in-patients (15 from each hospital) and 30 from out-patients, where the ratio of men and women was almost equal. Among the isolates, 93% were MDR; more than half of them (54%) were resistant to 10 antibiotics or more (highly resistant), while others were resistant to 3 to 9 antibiotics (regular MDR). The ratio of the highly resistant isolates was nearly three times higher at Imam Hospital and more than two times higher in in-patients. In contrast, regular MDRs were more than twice at Loghman Hospital, and although nonsignificantly, more prevalent among out-patients. Phylogroup B2 was the most common (52% of isolates), followed by F and E group (23% and 10% respectively). In phylogroup F, the ratio of highly resistant isolates was 2.5 times higher. In phylogroup A (3% of isolates) all were highly resistant. The isolates with higher antibiotic resistance formed weak or no biofilm. Logically, those that have not formed biofilm were all from Imam Hospital, and those that formed strong biofilm belonged to Loghman Hospital.

#### CONCLUSION

It seems that the more resistant UPECs are to antibiotics, the weaker biofilm they form. Although it might be obvious that the UPECs in hospitals are more resistant than the ones in community, this study shows that resistance pattern might depend more on the hospital itself.

**Keywords:** Urinary tract infection, *E. coli*, antibiotic resistance, multidrug resistant, nosocomial infection

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Optimized detection of *Salmonella typhimurium* using aptamer lateral flow assay

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## ABSTRACT

*Salmonella typhimurium* is a type of pathogenic bacteria that is crucial in medicine and the food industry, as it can cause food poisoning, such as typhoid or typhoid fever. However, diagnosing this bacterium can be challenging since its symptoms, including fever, diarrhea, and dizziness, are common in many diseases. Hence, a specific and effective diagnostic method is needed to identify *S. typhimurium* accurately. The aim of this study is develop a sensor immunoassay using aptamer-based lateral flow assay (LFA) to detect *S. typhimurium*. For this, anti- *Salmonella* aptamer conjugated with gold nanoparticles (GNPs), firstly for design of a nanobioprobe and as a signal probe, and used in LFA. The test involved a competitive format between the bacteria immobilized on the membrane and the bacteria present in the tested sample. In the following, the optimization of various factors affecting the aptamer LFA including the concentration of bacteria in stabilized form and into the sample, and the concentration of nanobioprop, was performed using the Taguchi test design method. The data showed that the optimal conditions for the LFA reaction was  $10^8$  cfu/mL of stabilized bacteria and  $1.5 \mu\text{g}/\mu\text{L}$  of nanobioprop concentration and under these conditions, the visual detection limit of *S. typhimurium* was estimated and calculated to be  $10^5$  cfu/mL. The reaction results are obtained within 20 minutes, and there were no significant cross-reactions with other food pathogens. In conclusion, the optimized aptamer-LFA diagnostic method provides a reliable, simple, and accurate tool for detecting *S. typhimurium*, and it can be used as a portable diagnostic kit for this bacterium in future investigation.

**Keywords:** Aptamer, Lateral flow assay, *Salmonella typhimurium*, Taguchi test design

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of contamination of traditional Babol ice creams with *Shigella* species containing the *Shigella flexneri* IcsA (VirG) gene

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Ice cream is one of the dairy products that, due to its nutritious ingredients, are a suitable environment for the activity of all kinds of microorganisms. Shigellosis is a type of infectious inflammatory colitis caused by one of the *Shigella* types. The purpose of this study is to evaluate the bacterial contamination of traditional ice creams in Babol city with *Shigella* species containing the *Shigella flexneri* IcsA (VirG) gene.

### MATERIALS AND METHODS

In this study, various types of ice cream including bread, glass, and funnel (20 Samples of each one = 60 samples) were randomly collected from the city of Babol in 2021 and transferred to the microbiology laboratory under sterile conditions while maintaining the cold chain. Selenite F culture medium, Hecton enteric agar was used to confirm the diagnosis of *Shigella* from microbiological tests. PCR was used to identify the gene (VirG). The frequency distribution table and t-test were used for statistical analysis ( $P < 0.05$ ) (2, 3).

### RESULTS AND DISCUSSION

The results showed that the percentage of contamination in funnel ice cream was 6.66% (4 samples), glass ice cream 8.33% (5 samples), and saffron ice cream 33.18% (11 samples). The results of the Polymerase chain reaction (PCR) test showed that none of the examined samples had the gene (VirG). A significant relationship was shown between contamination of ice creams with *Shigella* the *Shigella flexneri* IcsA (VirG) gene frequency ( $P = 0.001$ ).

### CONCLUSION

The results showed that the level of contamination of the traditional ice creams of Babol city was low and they did not have any gene (VirG). However, maintaining hygiene during the production and distribution of this sensitive product is a priority that can be achieved through training and strict health inspections.

**Keywords:** Contamination, Traditional Ice Creams, *Shigella flexneri* IcsA (VirG) gene

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The rate of *Salmonella* diarrhea in Holstein's calves in Golestan province and determining its antibiotic resistance pattern

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### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Losses of calves are significant in all parts of the world and have a special sensitivity due to their nutritional and economic value, so it is important to control the spread of diseases such as *Salmonella* diarrhea and treat them with appropriate antibiotics). This research aimed to survey the rate of *Salmonella* diarrhea in Holstein calves in Golestan province and determine its antibiotic resistance pattern in 2020.

#### MATERIALS AND METHODS

Using a sterile swab, 57 Holstein calves under 3 months of age with diarrhea were taken from the rectal area and fresh feces. The samples were cultured on a Salmonella-Shigella agar medium. Laboratory and microbiological methods were used to confirm Salmonella. The antibiogram test was performed using the disc diffusion method. Comparison of mean data was done using SPSS 19 software, one-way analysis of variance, and Duncan's test ( $p < 0.05$ ) (IR.IAU.BABOL.REC.1399.027).

#### RESULTS AND DISCUSSION

42% of the samples were infected with Salmonella. At the age of one month; 67%, two months old; 26%, and three months; 20% of the calves with diarrhea had Salmonella bacteria. Diarrhea decreased with increasing age ( $p < 0.05$ ). 21% of the calves with diarrhea weighing more than 90 kg had *Salmonella*. Diarrhea decreased with increasing weight ( $p < 0.05$ ). Salmonella samples were respectively resistant to; tetracycline (100%), cefazolin (95%), ampicillin (76%), trimethoprim- sulfonamide (75%), enrofloxacin, kanamycin and ciprofloxacin (each one 71%), amoxicillin (65%), gentamicin (43%) and ceftriaxone (33%). There was no significant difference between antibiotic resistance, age, and weight of calves ( $p < 0.05$ ).

#### CONCLUSION

The presence of diarrhea caused by salmonella was observed in the calves. The highest antibiotic resistance was against tetracycline and cefazolin. It is recommended to control the spread of diarrhea in livestock and to treat them quickly with appropriate antibiotics.

**Keywords:** *Salmonella*, diarrhea, Holstein calves, antibiotic resistance

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# In Silico Study on Immunogenicity of L3Omp34-L3OmpA and L3OmpA-L7BauA Constructs on LCL Scaffold against *Acinetobacter baumannii*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Acinetobacter baumannii* is a challenging Gram-negative pathogen known for causing difficult-to-treat infections. Vaccination offers the best defense against these infections, and selecting biologically vital proteins can enhance the efficacy of a multi-antigen recombinant vaccine. This study aimed to design optimized structures using selected OMP epitopes to improve the immune response against *A. baumannii*.

### MATERIALS AND METHODS

The loop-free portion of the C lobe of the TbpB protein from *Neisseria meningitidis* M982, known as LCL, was utilized as a scaffold to display conserved and exposed regions of *A. baumannii* OMP epitopes. Two combined structures, L3Omp34-L3OmpA and L3OmpA-L7BauA, were constructed using Loop3OmpA, Loop3Omp34, and Loop7BauA. Bioinformatics tools were employed to analyze various aspects of the constructs, including B-cell epitope prediction, antigenicity, secondary structure prediction, 3D modeling, and toxicity assessment.

### RESULTS AND DISCUSSION

The L3Omp34-L3OmpA and L3OmpA-L7BauA constructs exhibited promising characteristics. BepiPred2 predicted 100% and 94.5% linear B-cell epitopes, while Jpred4 indicated that 100% and 90% of epitopes were coiled. I-TASSER modeling revealed that 89% and 100% of selected loops were exposed on the construct's surface. The regions selected by VaxiJen were predicted to be antigenic. Although the constructs were potentially insoluble, as predicted by biotech software, the ToxinPred server indicated that they were non-toxic.

### CONCLUSION

Based on the results, the L3Omp34-L3OmpA and L3OmpA-L7BauA recombinant constructs show promise in enhancing immunoprotection against *A. baumannii* infections. These constructs hold potential as vaccine candidates for combating *A. baumannii* and warrant further investigation and experimental validation.

**Keywords:** *Acinetobacter baumannii*, Loop3OmpA, Loop3Omp34, Loop7BauA, vaccine candidate, LCL

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Comparison of Neutralization Potency across Passive Immunotherapy Approaches as Potential Treatments for Emerging Infectious Diseases

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The use of passive immunotherapy, either as plasma or purified antibodies, has been recommended to treat emerging infectious diseases (EIDs) in the absence of alternative therapeutic options. Here, we compare the neutralization potency of various passive immunotherapy approaches designed to provide the immediate neutralizing antibodies as potential EID treatments.

### MATERIALS AND METHODS

To prepare human plasma and purified IgG, we screened and classified individuals into healthy, convalescent, and vaccinated groups against SARS-CoV-2 using qRT-PCR, anti-nucleocapsid, and anti-spike tests. Moreover, we prepared purified IgG from non-immunized and hyperimmunized rabbits against SARS-CoV-2 spike protein. Human and rabbit samples were used to evaluate the neutralization potency by sVNT.

### RESULTS AND DISCUSSION

In comparison to all groups, the purified IgG of hyperimmunized rabbits had higher levels of neutralizing antibodies, with IC<sub>50</sub> of 11.48 µg/ml. Additionally, our results indicated a statistically significant positive correlation between the neutralization IC<sub>50</sub> value and the positive endpoint concentration of spike-specific antibodies.

### CONCLUSION

In conclusion, our study revealed that purified IgG from hyperimmunized animals has greater neutralization potency than other passive immunotherapy methods and may be the most suitable and quick treatment of EIDs.

**Keywords:** Emerging diseases, Passive immunotherapy, Plasma therapy, Neutralizing antibodies, Purified antibody

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### Molecular characterization of the *flaB2* gene of pathogenic *Leptospira* vaccinal serovars

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Leptospirosis is one of the most widespread zoonotic diseases which caused by pathogenic *Leptospira*. The flagellin proteins of pathogenic leptospires such as FlaB2 play essential role in pathogenesis of the disease. Therefore, a major challenge to develop an effective vaccine against leptospirosis is application of basic research on the flagellins of *leptospira* to improve vaccine development. The aim of this study was to analyse the nucleotide sequence of *flaB2* gene from vaccinal serovars of *leptospira* and phylogenetic comparison with clinical isolates.

#### MATERIALS AND METHODS

In this study, 5 vaccinal serovars of *Leptospira* and two non-pathogenic *Leptospira* serovars were used. The Leptospiral genomic DNA was extracted by standard Phenol-Chlorophorm method. The *flaB2* gene were amplified with PCR and specific primers. The nucleotide sequences of *flaB2* gene were analysed for their homology between them and other submitted sequences in the GenBank database using the BLAST and MegAlign software.

#### RESULTS AND DISCUSSION

The PCR product of *flaB2* gene was 1050 bp gene size in vaccinal serovars tested and it was observed that all 5 *leptospira* vaccinal serovars contained the *flaB2* gene. This gene was not observed in non-pathogenic serovars. In our study nucleotide sequencing results showed high similarity (100 %) among the *leptospira* vaccinal serovars and also isolates with sequences submitted in the GenBank.

#### CONCLUSION

The results concluded that the *flaB2* gene has high conservation between various *leptospira* serovars. The results suggested that *flaB2* gene may useful for preparation of recombinant antigen and candidate for recombinant vaccine and can also be used in an ELISA kit for the serodiagnosis of Leptospirosis.

**Keywords:** Leptospirosis, *flaB2* gene, Molecular characterization, Vaccinal serovars of *Leptospira*, Sequence analysis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Biofilm inhibition by *Lactobacillus rhamnosus* and *Lactobacillus paracasei* metabolites against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Controlling biofilm-related infections is a challenging issue due to increasing antibiotic resistance and high rate of infection recurrence after treatment. Probiotic Lactobacilli can act as microbial barriers against pathogenic bacteria. In the current research, the activity of two Lactobacilli against biofilm formation was investigated.

### MATERIALS AND METHODS

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), as well as the biofilm inhibition and destruction of the cell-free supernatant of *Lactobacillus rhamnosus* PTCC 1637 and *Lactobacillus paracasei* PTCC 431, were assessed against *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, MRSA and *Pseudomonas aeruginosa* PAO1 using the crystal violet assay. Additionally, the antimicrobial activity of the supernatants was measured by the agar well diffusion and the composition of the supernatants was detected by gas chromatography (GC).

### RESULTS AND DISCUSSION

The MIC of *L. paracasei*'s supernatant against *S. aureus*, *P. aeruginosa*, and MRSA was 12.5 mg/ml, and for PAO1 strain was 6.25 mg/ml. The lowest inhibitory concentration of *L. rhamnosus* for all tested strains was 12.5 mg/ml. The MBC of *L. paracasei*'s metabolites for *P. aeruginosa* and MRSA strains was 25 mg/ml, and for *S. aureus* and PAO1 strain, it was 12.5 mg/ml. Additionally, the MBC of *L. rhamnosus* strain for *S. aureus* and MRSA strains was 25 mg/ml and for *P. aeruginosa* and PAO1 strains was 12.5 mg/ml. Concentrations lower than MIC (sub-MIC) of both strains significantly inhibited biofilm formation in the tested strains. Furthermore, both supernatants, at concentration of 3-5 times greater than MBC significantly destructed of the formed biofilm.

### CONCLUSION

[Both *L. rhamnosus* and *L. paracasei* strains had a significant inhibitory and destructive effect on the biofilm of the tested strains. The most antimicrobial effect was related to lactic acid and acetic acid.

**Keywords:** biofilm, probiotic, antibiotic resistance, *L. rhamnosus*, *L. paracasei*, *S. aureus*, *P. aeruginosa*

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# Anti-inflammatory properties of pigment extracted from newly isolated halophilic bacterial strain

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Various microorganisms are able to synthesize pigments which frequently has been reported their antioxidant properties. But recently, researchers have been interested in evaluating other pharmaceutical effects of these natural products, e.g. anticancer, antimicrobial, antiviral and antidiabetic attributes. In this study we evaluated the anti-inflammatory activity of halophilic bacterial pigments since nonsteroidal anti-inflammatory drugs have certain side effects. Greater metabolic flexibility of halophilic bacteria, lower nutrient requirements, genetic machineries of adaptation to harsh conditions such as dryness, sun radiation and high ionic strength make them a promising candidate and hope for drug discovery.

### MATERIALS AND METHODS

Out of 34 halophilic colored bacteria, the extracted pigments from a cocci bacterium designated as strain AS showed anti-inflammatory activity and were measured using human red blood cells (RBCs) membrane stabilization method via hypotonic solution- induced hemolysis. 100 to 1000 µg/ml extracted pigments were mixed with RBCs suspension and membrane rupture of these cells monitored by UV-Vis spectrophotometer. The type of extracted pigments is estimated by spectrophotometric analysis and Thin Layer Chromatography (TLC). Molecular identification and biochemical characterization of the pigmented isolate was carried out

### RESULTS AND DISCUSSION

The membrane RBCs were used for inflammation inhibition evaluation as their membrane behaves similarly to the lysosomal membrane. Our extracted pigments showed a significant potent on human erythrocytes, adequately protecting them against the hypotonic solution. The extracted pigment's hemolysis inhibition %, ranged from %11.08 to %69.35. TLC analysis showed 3 fractions and more analysis is being conducted. Phenotypic characterization and phylogenetic analysis based on 16S rDNA sequence comparisons indicated that this strain was a member of the genus *Salinicoccus*.

### CONCLUSION

This pigment as a natural product could be an alternative to nonsteroidal anti-inflammatory drugs that have negative side effects and should be avoided by people with certain medical problems.

**Keywords: halophilic bacteria, bacterial pigments, Anti-inflammatory activity**

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# Frequency of Seropositive Cases of Brucella among Suspected Patients in Sari, during 2021-2023

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Brucellosis is a bacterial disease that is endemic in Iran due to extensive animal husbandry, especially in Mazandaran. If the disease left untreated, it advances to the chronic phase with complications such as osteomyelitis, bone pain, and jaundice. Laboratory diagnosis of brucellosis is often accomplished by serologic testing. The aim of this study was to investigate seropositivity of Brucella in suspected patients, referred to the laboratory, in Sari.

### MATERIALS AND METHODS

In a cross-sectional study, blood samples were collected from 1633 suspected brucellosis individuals who were referred to the laboratory. Wright, 2-Mercaptoethanol (2-ME), and Coombs-Wright tests were conducted for them. People who had an antibody titer in Wright or Coombs-Wright test higher than 1:160 or 2-ME more than 1:80; were considered seropositive. Individuals were categorized based on age and gender.

### RESULTS AND DISCUSSION

In this study, 1125 people (68.9%) were female. 48 people (2.94%) were seropositive. The average age of seropositive individuals was  $49.8 \pm 17.2$  years. there was not a significant relationship between the seropositivity and gender, (females: 2.67%, males: 3.54%), but in the range between 30 to 39 years the correlation was significant (females: 1.10%, males: 6.54%). 30 seropositive individuals (62.5%) were referred to the laboratory in the first half of the year.

### CONCLUSION

According to the results obtained from this study, brucellosis is still an important public health problem in Iran. Middle age Males are more susceptible to brucellosis and the probability of contracting the disease increases in the first half of the year.

**Keywords: Brucellosis, Prevalence, Serological diagnosis**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Correlation between *clumping factor A* gene expression in biofilm formation and antibiotic resistance among *Staphylococcus aureus* isolated from urine samples of Imam Khomeini Hospital, Tehran

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## ABSTRACT

### BACKGROUND & OBJECTIVE

*Staphylococcus aureus* causes problem in hospitals and in recent years, it has emerged as a serious agent acquired from the environment. One of the capabilities of *S. aureus* is the formation of biofilm, in which bacteria can exchange antibiotic resistance genes among themselves and increase the virulence of other strains of this species (*S. aureus*). A surface protein attached to the cell wall in *S. aureus clumping factor A* is a virulence factor in various staphylococcal infections.

### MATERIALS AND METHODS

In this study, after the Urea analysis (UA) test, the urea culture test was applied to the blood agar and Baird-Parker Agar culture media from the infectious urine samples in Imam Hospital in Tehran, *S. aureus* isolates were identified. Finally, molecular method was used for confirmation of identified isolates. The microtitre plate method was performed to determine the biofilm formation ability. Disk diffusion method was also used for the profiling the antibiotic resistance of the isolates.

### RESULTS AND DISCUSSION

In the results of this study, out of 160 urinary clinical samples, 45 samples were positive *S. aureus* among which 42 isolates expressed the *clfA* gene. Moreover, 39 isolates had the ability to form biofilms in the *in vitro* environment. Also, among these 42 isolates, the highest rate of antibiotic resistance (88%) was against penicillin and the lowest resistance rate (16%) was against cefoxitin. After checking with SPSS software and chi-square, there was a significant relationship between gene expression and biofilm production with antibiotic resistance ( $P < 0.05$ ).

### CONCLUSION

Due to the repeated use of antibiotics such as beta-lactams, Especially in respiratory infections and pharyngitis, the resistance of *S. aureus* bacteria is increasing strongly, and the formation of biofilm and virulence factors such as *clfA* and *clfB* cause concern to the World Health Organization for treatment, especially for people who they have sepsis or toxemia.

**Keywords:** *Staphylococcus aureus*, *clumping factors*, coagulase, biofilm, microtitre plate, disk diffusion, virulence factors

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Adherence inhibition by anti-Oma87 antibodies of *Acinetobacter baumannii* to A549 cell line

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Acinetobacter baumannii* is a nosocomial pathogen that has stunned the world and poses a significant threat to the global healthcare system, most notably in ventilator-associated pneumonia, bloodstream infections, urinary tract infections, and meningitis. Adhesion to target cells is essential for a pathogen. This is established with the expression of virulence factors. *A. baumannii* stems from its ability to adhere and colonize biotic and abiotic surfaces, employing a change of strategy currently dubbed 'persist and resist' rather than the traditional toxin expression of other pathogens. This, coupled with an equally confounding capacity to survive in normally unfavorable conditions makes *A. baumannii* a formidable pathogen.

### MATERIALS AND METHODS

rOma87 was expressed, purified, and injected into groups of BALB/c mice. The antibody titer was measured by the indirect ELISA. Adhesion and internalization of *A. baumannii* strains were studied in A549 cell line.

### RESULTS AND DISCUSSION

Oma87, a conserved and potent immunogen of *A. baumannii*, significantly inhibited adhesion to and internalization in A549 cells of *A. baumannii* strains.

### CONCLUSION

Monovalent vaccines containing conserved antigens offer a satisfactory level of protection against *A. baumannii* infections.

**Keywords:** *Acinetobacter baumannii*, Adherence, Cytotoxicity, Oma87, Internalization

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluate of tetracycline-resistant genes among Shiga-toxin-producing *Escherichia coli* (STEC) strains from diarrheic human cases in Kerman

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

Infectious diarrhea, one of the most common syndromes of gastrointestinal infections, is caused by several bacterial agents. *Escherichia coli* (*E. coli*) could be considered one of the most prevalent bacterial species of gastrointestinal infections. This study aimed to evaluate the presence of tetracycline-resistant genes among Shiga-toxin-producing *E. coli* (STEC) strains from diarrheic human cases in Kerman, southeast of Iran.

##### MATERIALS AND METHODS

In this study, 39 Shiga-toxin-producing *E. coli* (STEC) strains from people with diarrhea were screened for the presence of tetracycline genes by conventional polymerase chain reaction (PCR).

##### RESULTS AND DISCUSSION

Out of the 39 analyzed samples, one sample (0.39%) were positive for *tatA* gene, and 10 (3%) for *tetB* gen.

##### CONCLUSION

This study showed the presence of antimicrobial resistance genes encoding *tetA* and *tetB* in diarrheal strains of *E. coli* in southeast Iran. Therefore, there is a need to increase surveillance in hospitals and reduce indiscriminate antibiotic prescribing to reduce the prevalence of antibiotic-resistant *E. coli*.

**Keywords:** *Escherichia coli*, Diarrhea, Antibiotic resistance genes, tetracycline genes.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### An inexpensive method for screening and dereplication of bioactive metabolites of *Leuconostoc mesenteroides* UTMC 3821

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Members of *Leuconostoc* genus are producers of diverse compounds that have been used in various food and medical industries. These bacteria produced more than 30 bacteriocins with various activity against various g<sup>+</sup> and g<sup>-</sup> bacteria. However, antibacterial activity against *Salmonella* has not been reported previously. *Leuconostoc mesenteroides* subsp. *mesenteroides* UTMC 3821 was isolated, previously. In the current research, to avoid re-isolation of previously discovered bioactive compounds, we developed a differential method based on the physicochemical and biological characteristics of previously discovered bacteriocins of *Leuconostoc* species. This method was used to differentiate the bioactive compounds of the isolate.

#### MATERIALS AND METHODS

The UTMC 3821 strain was cultured in MRS broth and incubated at 28 °C for 24 h. After removing the biomass by centrifugation at 4000 rpm for 15 min, the supernatant was extracted with ethyl-acetate. The solvent was evaporated at reduced pressure and temperature, and the antimicrobial activity of the metabolite was examined using the agar diffusion method against seven test microorganisms. Additionally, the effect of various temperatures (50°C, 60°C, 70°C, 100°C, and 120°C) and times (15, 30 minutes) on the bioactivity of the metabolites against *Salmonella* was detected.

#### RESULTS AND DISCUSSION

The metabolite(s) of *Leuconostoc mesenteroides* UTMC 3821 are thermotolerant (120 °C, 30 min) and active against *Salmonella*, *Pseudomonas*, and some other g<sup>+</sup> and g<sup>-</sup>-bacteria, unlike other metabolites of members of *Leuconostoc* genus.

#### CONCLUSION

The method introduced in this research is an inexpensive and applicable method for differentiating and screening *Leuconostoc* bacteriocins. Considering the unique characteristics of *Leuconostoc mesenteroides* subsp. *mesenteroides* UTMC 3821 metabolites, further research is encouraged to determine whether the metabolite(s) can be applied in food industry and aviculture.

**Keywords:** Bacteriocins, *Lactic acid bacteria*, *Leuconostoc*, *Salmonella*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Contamination of Cockroaches (Insecta: Blattaria) by *Escherichia coli* : A Systematic Review and Meta-analysis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The human intestine inhabitant and an environmental bacteria *Escherichia coli* (*E. coli*) which can be pathogenic or nonpathogenic as the normal microflora of warm-blooded animals, including humans. *E. coli* is a rod-shaped, Gram-negative bacterium in the family Enterobacteriaceae. *E. coli* is more than harmless intestinal inhabitant, it can be highly versatile, and frequently deadly, pathogen which cause various infections like urinary tract infection, sepsis/meningitis, enteric/diarrheal disease, arteriosclerosis, hemolytic uremic syndrome. Insects like cockroaches often carry microorganisms especially pathogenic organisms and their medical importance in the spread of infections cannot be ruled out. Therefore a systematic review and meta-analysis was conducted to investigate the prevalence Contamination of Cockroaches (Insecta: Blattaria) by *Escherichia coli*.

### MATERIALS AND METHODS

A systematic literature search was conducted until the end of 2023 in electronic databases, including Web of Science, PubMed, Scopus and Embase. Data analysis was performed with the 'metaprop program' in STATA statistical software version 11.0 (Stata, College Station, TX, USA).

### RESULTS AND DISCUSSION

In this study 1344 article, included the studies published for 2010 until now through two databases, namely, Scopus, Web of Science and PubMed identified, 33 studies were included in the scoping review.

### CONCLUSION

This statistical analysis indicates that the bacterial contaminants of the external cockroach body parts are potentially more harmful than from internal surfaces, and secondly, the bacterial contaminants of cockroaches in hospital environments are potentially more harmful than from other human environments. The survey indicated that the bacterial contaminant species of cockroaches appear to be mostly multiple drug resistant. The challenges of cockroaches as being potential vectors of pathogenic or opportunistic agents of human infections are discussed.

**Keywords:** Cockroaches, *Escherichia coli*, Bacterial contamination, Systematic Review, Meta-analysis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### A review of recent improvement in allogeneic hematopoietic cell transplantation for acute myeloid leukemia

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#### ABSTRACT

At present, allogeneic stem cell transplantation (allo-HSCT) is a very effective and unique method for the treatment of some malignant and non-malignant blood disorders. However, therapeutic approaches with the potential to reduce disease relapse include advances in induction chemotherapy, new preparative regimens, as well as the evolving concept of post-transplant maintenance. Despite these advances, disease recurrence remains a major cause of graft failure. Current evidence suggests that allo-HSCT is increasingly offered to older patients, including those above 70 years of age with acute myeloid leukemia (AML). On the other hand, the intensification of standard drug dosage and safer allogeneic methods of HSCT allow a larger proportion of patients to achieve a sustainable recovery. Therefore, there is a need to better understand the underlying mechanisms as well as to develop new strategies for prevention and treatment in order to improve allo-HSCT outcomes. In this study, we reviewed recent advances in allo-HSCT in patients with AML.

**Keywords:** Acute myeloid leukemia, allogeneic hematopoietic cell transplantation (allo-HSCT)

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular identification and antifungal susceptibility profiles of *Candida* species from Iranian Northwest hospital isolates

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

There is an increasing incidence of life-threatening systemic fungal especially caused by *Candida* species. Limited therapeutic option due to the increasing level of resistance to antifungal agents, calls for the prompt identification of drug-resistant isolates. In order to identify the most common *Candida* species isolate from clinical cases and to optimize antifungal therapy against them, we performed this experimental study.

### MATERIALS AND METHODS

The study was done on 198 clinical specimens collected from proven cases of Hospital Acquired Infections (HAIs) between August 2018 and September 2020. Direct microscopic examination and culture were performed for the detection and isolation of the *Candida* species. PCR-RFLP and real-time PCR were performed for confirmation and molecular typing of *Candida* isolates. Antifungal susceptibility testing using CLSI BMD MIC (M27-A2) guidelines was performed on the isolated *Candida* species.

### RESULTS AND DISCUSSION

The results of molecular identifications showed that, 54(58%) had a fungal infection. Out of the total fungal isolates of HAIs, 66.6% (36/54) belong to *Candida* species. including *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. Krusei*, *C. guilliermondii* and *C. parapsilosis*. Our results of antifungal susceptibility testing revealed that 5 out of 15 *C. albicans* and 1 out of 3 *C. dubliniensis* clinical isolates were resistant to itraconazole (MIC 24h) and only case of *C. tropicalis* was resistant after 48 hours.

### CONCLUSION

We found that *C. albicans* as the commonest pathogenic yeast in hospitals decreased susceptibility to itraconazole (a routinely used antifungal drug). Molecular identification of *Candida* species and their resistance patterns is essential for optimized patients' management and reduction in unnecessary drug overuse.

**Keywords:** *Candida* identification, Drug sensitivity, Hospital, Antifungal Agents

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Identification of Carbapenem Resistance Genes in *Escherichia coli* Isolated from *Blattella germanica* by Dot Blot Assay in Hamadan Hospitals, Iran – 2018

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Today, one of the problems of health systems is the presence of cockroaches in hospitals as insects that move freely in and out of the hospitals and are infected with pathogenic bacteria. The aim of this study was to identify carbapenem resistance genes in *Escherichia coli* isolated from *Blattella germanica* by dot blot assay in Hamadan hospitals in the west of Iran.

#### MATERIALS AND METHODS

A total of 109 *B. germanica* from April to September 2018 were collected from ICUs of different hospitals in the Hamadan province, located in western Iran. The *B. germanica* were identified using reliable taxonomic keys by an expert in the Department of Entomology, insectarium Hamadan University of Medical Sciences, Iran. The antimicrobial susceptibility test was determined by disk diffusion. The dot blot assay was used to identify resistance genes in *E. coli* isolated from *B. germanica*.

#### RESULTS AND DISCUSSION

Out of 109 *B. germanica* samples collected from ICUs of different hospitals in Hamadan, 31 samples (28.44%) were positive for *E. coli*. The highest frequency of antibiotic resistance against ampicillin (100%) and the lowest resistance to imipenem was observed in two isolates (6.45%). The frequency of genes among *E. coli* isolates in *B. germanica* was as follows: *bla* NDM (4 isolates: 3.66%), *bla* OXA-48 (one isolate: 0.92%), and other studied genes were not observed in any of the strains.

#### CONCLUSION

Cockroaches are an important factor in transmitting *Enterobacterales* and multidrug-resistant (MDR) strains. Therefore, effective preventive and control measures are needed to reduce vector-borne diseases.

**Keywords:** Antimicrobial drug resistance, *Blattella germanica*, Dot blot assay, *Escherichia coli*, German cockroaches

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Identification of most important pathogenic *Aspergillus* species using the molecular method, PCR-SSCP

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

SSCP as a molecular PCR based method has been interested for rapidly identification of fungal species. This method was previously used for the other aims including analysis of mutations as well as identification and discrimination between some organisms.

In the present study, we tried to use the free digestion method of SSCP for discriminating *Aspergilli* in the species level considering polymorphism patterns between them.

### MATERIALS AND METHODS

Our major strains isolated from clinical and environmental specimens collected from four Iranian educational hospitals in Urmia. Some other specimens obtained from Medical Mycology lab, Urmia University of Medical Sciences. All of the *Aspergillus* isolates, identified by using four *Aspergilli* differential media including, CZA, CYA, CYA20S and MEA, following by macroscopic and microscopic examinations. In the molecular assay, we extracted DNA manually using Glass beads and Phenol chloroform method, followed by PCR amplification of ITS2 region and then thermal denaturizing DNA to make single stranded DNA. Finally, we compared differentiate patterns of electrophoresis bands.

### RESULTS AND DISCUSSION

During an 18-month started from July 2018, we obtained 205 *Aspergillus* isolates including 11 species, from the hospital clinical and environmental specimens. Hospital sources included clinical (25%) and environmental (75%) isolates. Our findings of SSCP method included: *A. nidulans*, (*A. fumigatus*, *A. niger*) as a category and (*A. flavus*, *A. terreus*, *A. ochraceus*) as the other category.

### CONCLUSION

SSCP as a rapid and simple molecular method enabled us to identify most important pathogenic *Aspergillus* species.

**Keywords:** *Aspergillus*, identification, SSCP

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prediction of death in burn patients with septic shock with *Pseudomonas aeruginosa* using machine learning based techniques

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa* is an opportunistic bacterial pathogen and important cause of wound infection and sepsis in burn patients. In this study, using machine learning (ML) a new and valuable step has been taken to predict the risk of death in burn patients with septic shock infected with *P. aeruginosa*.

### MATERIALS AND METHODS

In this study, data from the records of burn patients with septic shock infected with *P. aeruginosa*, including demographic information (such as age, gender), percentage of burns and other clinical variables of these patients in the hospital of Rasht province, will use to predict the risk of death using two ML methods, SVM (Support Vector Machine) and ANN (Artificial Neural Network). The data will divide into two training and testing categories. AUC index (Area under curve) will used to evaluate the accuracy of the models.

### RESULTS AND DISCUSSION

In this research, a new and valuable step in predicting the risk of death due to *P. aeruginosa* sepsis in burn patients and also identifying antibiotic resistance in patients with septic shock which infected with *P. aeruginosa*.

### CONCLUSION

The advances achieved in the field of artificial intelligence (AI) today witness different applications in diagnostic/preventive medical sciences. Such research will be of great help to the decision-makers in the field of treatment and health and will greatly reduce the costs of treatment and diagnosis.

**Keywords:** *Pseudomonas aeruginosa*, Burn Patients, Machine Learning, Artificial Intelligence, Data Mining

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Synthesis of Copper oxide nanoparticles and study the antifungal properties

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nanomaterials are known as new antimicrobial agents. Their high surface-to-volume ratio and their unique physical and chemical properties increase their contact with microbes. The antimicrobial effects of nanoparticles on fungi, bacteria and Viruses have been well studied. Copper nanoparticles, due to their physical, chemical, and biological properties, have capacity to inhibit growth of the different microorganisms.

### MATERIALS AND METHODS

In this study, copper oxide nanoparticles were synthesized by microwave method and analyzed with XRD, EDX, SEM, FTIR, and UV-vis methods in order to investigate their physical and chemical properties. Antifungal effects of copper oxide nanoparticles on *Fusarium solani* fungus by Agar well diffusion method with concentrations of 12.5 to 100 mg were evaluated.

### RESULTS AND DISCUSSION

The results demonstrated that this fungus shows relatively more sensitivity in concentrations of higher than 50 mg/ml, as the growth inhibition zone at concentration of 100mg/ml was 18 mm.

### CONCLUSION

Therefore, copper oxide nanoparticles can be introduced as a strong antimicrobial agent.

**Keywords:** Nanoparticles, Copper oxide, Microwave synthesis, Antifungal

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Cellulose production from bacteria isolated from rotten fruit jam

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The purpose of this study was to isolate and identify bacteria capable of producing bacterial cellulose and to optimize the conditions for the production of bacterial cellulose that can be useful for commercialization and industrial applications.

#### MATERIALS AND METHODS

In this study, the bacterial isolates obtained from rotten blackberry jam. A sample of rotten blackberry jam was prepared and cultured on Hestrin-Schramm's agar medium. In this study, a strain with maximum ability to produce cellulose was isolated from rotten jam samples and identified as *Rhodococcus* sp. After the purification of cellulose, enzymatic digestion by cellulase enzyme was used to confirm the presence of cellulose. Optimization studies were carried out for process parameters such as inoculum density, temperature, pH, stirring and carbon and nitrogen sources.

#### RESULTS AND DISCUSSION

The strain produced 2.43 g/L cellulose in 6 days under optimal growth conditions of temperature (30 °C), pH (6.0), glucose (2%), yeast extract (0.5%) and peptone (0.5%). These findings are for continuous improvement of cellulose synthesis by *Rhodococcus* sp. for the production of cellulose on an industrial scale. Scanning electron microscopy also showed that the cellulose produced by this bacterium had a fibrous structure and micron-sized pores.

#### CONCLUSION

The results of the present study showed that *Rhodococcus* strains have the ability to produce cellulose. Cellulose produced by bacteria is preferred for use in various industries due to its higher purity than plant cellulose. The product obtained from this bacterium can be used as a cosmetic and medical material.

**Keywords:** Bacterial cellulose, Optimization strategies, Applications.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# One-pot preparation of laccase@yttrium phosphate hybrid nanostructures as a novel antibiofilm agent

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

A number of strategies have been developed in the last decade to inhibit biofilm formation on biotic and abiotic surfaces. Today, laccases are known to stand as important antibiofilm agents because these molecules can inhibit biofilm formation in an eco-friendly manner. A detailed antibiofilm mechanism of laccase includes penetrating the microbial membrane and oxidizing essential proteins, enzymes, or other cellular sites. For biofilm removal, enzyme immobilization provides excellent stability, high reusability, and better efficiency. In this regard, the use of hybrid nanostructures (HNSs) for enzyme immobilization has gained significant attention due to the ease of preparation and enhanced catalytic activity. Herein, the novel hybrid nanoflowers consisting of yttrium (III) ions combined with laccase were prepared and their antibiofilm activity was evaluated against some pathogenic bacteria.

### MATERIALS AND METHODS

The hybrid particles were synthesized in an aqueous solution using yttrium phosphate ( $Y_3(PO_4)_2$ ) as the inorganic component and laccase as the organic constituent. Then, the effects of reaction parameters on the formation of the enzyme-embedded hybrid nanoparticles were investigated. Furthermore, the antibiofilm activity of the prepared HNSs was evaluated against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. Finally, the biofilm formation in the presence of the free enzyme and laccase@YPO<sub>4</sub>•HNP was visualized by crystal violet staining and Confocal Laser Scanning Microscopy (CLSM).

### RESULTS AND DISCUSSION

The free laccase did not significantly prevent biofilm formation even increasing concentration up to 120 U L<sup>-1</sup>. Laccase@YPO<sub>4</sub>•HNPs effectively inhibited *E. coli*, *S. aureus*, and *B. subtilis* biofilms at concentrations of  $\leq 60$  U L<sup>-1</sup>. According to the crystal violet staining and CLSM images, the total cell coverage for the biofilms significantly declined compared to the control following the treatment with laccase@YPO<sub>4</sub>•HNPs.

### CONCLUSION

Laccase@YPO<sub>4</sub>•HNPs could be considered a novel, eco-friendly, and antibiofilm heterogeneous biocatalyst for medicine and industry.

**Keywords:** Laccase; Yttrium phosphate; Antibiofilm; Hybrid nanostructures

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Frequency of Enteropathogenic *Escherichia coli* strains isolated from children with diarrhea in Bushehr

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Enteropathogenic *E. coli* (EPEC) is associated with outbreaks of infantile diarrhea, and is a contributor to diarrheagenic diseases in human populations around the world. All EPEC strains contain the *eaeA* gene that encodes intimin for attachment to epithelial cells, differentiate from STEC by absence of the shiga toxin (*stx*) gene, and also classified into typical and atypical strains based on the presence or absence of bundle-forming pilus (*bfpA*) gene. Purpose of the current study was to find out the frequency of EPEC strains by PCR molecular method in Bushehr.

#### MATERIALS AND METHODS

A total of 165 *E. coli* strains isolated from diarrheic children less than 10 years were examined. The specimens collected from two hospitals from November 2021 to April 2022 in Bushehr province. All *E. coli* isolates were identified by Gram stain and standard biochemical tests such as TSI, motility, indole, simon citrate and urease. Total DNA was extracted by boiling method. *E. coli* isolates were confirmed by detection of *uidA* gene using PCR. Determination of EPEC isolates was analyzed using by PCR for the *eaeA*, *stx1*, *stx2* and *bfpA* genes.

#### RESULTS AND DISCUSSION

Out of these isolates, EPEC were detected in 9 (5.45%) of children by PCR, which harbored both *uidA* and *eaeA* genes. Additionally, all isolates have been checked for *Stx1* and *Stx2* genes. Males in this study constituted 58.2%, and females were 41.8%. EPEC isolates were then tested by PCR-based targeting of *bfpA* gene to differentiate typical and atypical EPEC. All EPEC strains displayed *eaeA*<sup>+</sup>, *stx1/stx2*<sup>-</sup>, *bfpA*<sup>-</sup> genotype as atypical EPEC isolates. Any isolate wasn't determined as typical EPEC strain with *eaeA*<sup>+</sup>, *stx1/stx2*<sup>-</sup>, *bfpA*<sup>+</sup> genotype in present study.

#### CONCLUSION

The findings of current study indicated atypical EPEC to be dominant subtype and should be considered in diagnosis of diarrhea among children. PCR method can be a reliable, fast and sensitive alternative applied to identify the EPEC strains, and it is recommended that these strains are detected by this molecular method.

**Keywords:** Enteropathogenic *Escherichia coli*, Diarrhea, PCR.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluating the Genetic Diversity of *Helicobacter pylori* Isolates in Patients Suffering from Gastritis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Helicobacter pylori* infections vary in severity and virulence in different populations for various reasons. There are different *H. pylori* strains with varying degrees of virulence. The genetic diversity of *H. pylori* strains in gastritis patients in different areas has not been well understood. This study aimed to evaluate the prevalence rate and different genotypes of *H. pylori* strains in clinical specimens of patients with gastritis in Ilam, Iran.

### MATERIALS AND METHODS

Saliva and gastric biopsy samples were collected from 81 patients (55 males and 26 females in the age range of 20 to 90 years) referring to Ilam medical centers. After DNA extraction, the prevalence of *H. pylori* as well as *vacA*, *cagA*, and *ureC* genes was evaluated using PCR, and then each *vacA*-positive sample was further evaluated for *m1m2* and *s1s2* variants.

### RESULTS AND DISCUSSION

The *cagA* and *vacA* genes were found in 27 (71%) and 36 (94.7%) *H. pylori*-positive samples, respectively. The *cagA* gene was detected in patients with gastric pain (44.4%) and anorexia (18.51%). Also, the results showed that the *vacA* *s2m2* genotype and *m2* allele were present in 32.9% of *H. pylori* isolates. Moreover, *s2m2* and *s1m2* genotypes were detected in 42.1 and 26.3% of *vacA*-positive samples, respectively. The lowest frequency was related to the *m1* allele (17.18%).

### CONCLUSION

This study results indicate a plausible relationship between the presence of some genotypes of *H. pylori* and the progression of gastritis, suggesting these markers as promising biomarkers to predict the disease severity.

**Keywords:** *Helicobacter pylori*, Gastritis, Genotyping, *vacA*, *cagA*, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Synthesis of nickel oxide nanoparticles and study the antifungal properties

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

A large number of diseases and infections caused by microorganisms have been reported worldwide. It is very important to control their spread and fight against them. Nanotechnology is a science which currently used in various industries such as medicine, chemistry, and biological sciences. One of the branches of this new technology is synthesis of nanoparticles by different methods.

### MATERIALS AND METHODS

In this study, nickel oxide nanoparticles were synthesized by hydrothermal method and analyzed by XRD, EDX, SEM, FTIR, and UV-vis methods to examine the physical and chemical features of these nanomaterials. In order to examine the antimicrobial activity of these nanoparticles, *Fusarium solani* fungus was used by Agar well diffusion method with concentrations of 12.5 to 100 mg.

### RESULTS AND DISCUSSION

The evaluation of the antifungal effects of nickel oxide nanoparticles showed that *Fusarium solani* was not sensitive to these concentrations and the zone of growth inhibition was insignificant.

### CONCLUSION

It can be said that nickel oxide nanoparticles which have been synthesized by hydrothermal method indicated an insignificant antifungal effect against this standard strain.

**Keywords:** Nanoparticles, Nickel oxide, Hydrothermal synthesis, Antifungal

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Decolorization of some reactive azo dyes by bacteria isolated from factories wastewater

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#### ABSTRACT

The presence of azo dyes in the environment is an issue of major concern since they are highly recalcitrant and toxic. Azo dyes are extensively used in textile industries, and account for more than 50% of the synthetic dyes used worldwide. Reactive azo dyes account for more than half of global dye production due to their ease of application, brilliant colors, and reactive units that quickly attach to fabrics. Releasing azo dyes into water bodies results in aesthetic problems and deteriorates water quality. This project described the degradation of three commercial model reactive dyes C.I.Reactive Yellow 160 (RY160), C.I.Reactive 195 (RR195) and C.I.Reactive 122 (RO122). For this purpose, wastewater samples were collected from three sites to isolate dye-decolorizing bacteria. The activity tests for microbial decolorization were performed in the culture medium containing 50 mg L<sup>-1</sup> individual dyes using incubator shaker at 30°C and 120 rpm. About every 7 days the culture was transferred to fresh medium. After 3, 5, and 7 days the ratio of decolorization was recorded by measuring the absorbance of the sample. The results show a decrease in absorbance indicates color removal by bacteria.

**Keywords:** Bacterial Decolorization, Azo Dye, Reactive, Wastewater

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### A review of the biological effects of myco-biopolymers

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The biopolymers are well known and many research and development activities are related to the use of biopolymers in clinical science, medical engineering, biotechnology and industry. Biopolymers obtained from renewable sources due to Versatility, biocompatibility, and degradability are very important in various industries, and antimicrobial, anticancer, wound healing and dressing effects, prebiotic effects, and other effects of myco-biopolymers are mentioned in this article.

#### RESULTS AND DISCUSSION

Nowadays, natural biopolymers derived from plant and fungi bases are very considered; Because it is non-toxic and biodegradable and this feature increases their potential application in biopolymers. Also, these macromolecules have very strong antioxidant, prebiotic and anticancer effects; In such a way that their antioxidant mechanisms mainly include the regulation of signal transmission pathways, the activation of enzymes and the elimination of free radicals, which in turn affect their anti-cancer properties. Cell walls that have a common chemical structure and are composed of homo and heteropolysaccharides, protein, protein-polysaccharide complexes, lipids, melanin and chitin polysaccharide chains are used in biopolymers with a fungal base. These macromolecules are known as myco-biopolymers, which have various applications in packaging, filtration, drug delivery, medical implants, pharmaceutical industries, wound and tissue repair.

#### CONCLUSION

Myco-biopolymers have many industrial and medical applications due to their good properties. On the other hand, considering the lack of food and maintaining food safety, researchers are of the opinion that food supplements should be supplied from other sources, especially fungi and algae, instead of plant and animal sources.

**Keywords: Biopolymer, Biology, Myco-Biopolymer, Fungus**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation and evaluation of probiotic properties of bacteria isolated from the medicinal plants of the *Asteraceae* family of Tehran and Karaj regions

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Probiotics are beneficial live microorganisms that promote host health when consumed in sufficient quantities. Medicinal plants, particularly those belonging to the *Asteraceae* or *Compositae* family, are a potential source for isolating probiotic bacteria. In addition, the rhizosphere, the ecological niche surrounding plant roots, is an important area for identifying probiotics due to the competition between resident microorganisms for growth factors.

### MATERIALS AND METHODS

A total of 204 Gram-positive bacteria strains were isolated from the aerial parts and soil surrounding the rhizosphere of genera belonging to *Achillea*, *Artemisia*, *Cichorieae*, *Echinops*, *Onopordum*, *Centaurea*, *Gundelia*, *Carduus*, *Jurinea*, *Tanacetum*, *Cichorium*, *Echinacea*, and *Grindelia*. These strains were cultured on MRS and TSA. In vitro assessments were conducted to determine the probiotic potential of the isolated bacteria, including hemolytic activity, resistance to gastric acid, resistance to bile salts, and resistance to pepsin and trypsin enzymes. Finally, 16S rRNA analysis was performed using primers 27 F and 1492 R for identification.

### RESULTS AND DISCUSSION

Based on the tests, a strain isolated on TSA medium from the rhizosphere soil of *Tanacetum vulgare* was subjected to molecular analysis and identified as *Bacillus licheniformis* through BLAST analysis. The strain exhibited characteristics such as non-hemolytic activity, ability to survive at pH 2.5 and 4, growth in a medium containing 0.3% bile salt, and growth in media containing 0.2% pepsin and trypsin.

### CONCLUSION

Medicinal plants and rhizosphere soil are among the resources that have been less considered for the isolation of probiotic bacteria. Therefore, the investigation, isolation, and identification of probiotic strains from the *Asteraceae* family have great importance in various industries.

**Keywords:** Probiotic bacteria, *Bacillus*, Medicinal plants, rhizosphere soil, *Asteraceae*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Serological survey of H<sub>5</sub>, H<sub>7</sub> and H<sub>9</sub> subtypes of Avian Influenza Viruses in human population related to poultry industry

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Influenza viruses are an acute respiratory, highly contagious and zoonotic disease which belong to the Orthomyxoviridae family. The H<sub>9</sub> subtype is an avian pathogenic influenza virus that its outbreak frequently occurs in poultry farms of Iran. As well, H<sub>5</sub> and H<sub>7</sub> subtypes are a highly pathogenic avian influenza subtype which causes high mortality in poultry and wild birds. Some subtypes of influenza viruses can transmit to human from birds and the antigenic shift is common among these viruses.

### MATERIALS AND METHODS

This study was carried out to determine antibodies to H<sub>5</sub>, H<sub>7</sub> and H<sub>9</sub> subtypes of avian influenza virus in different human population related to poultry industry in Ardabil area, northwest of Iran. In this survey, 105 blood samples were collected from poultry vaccinators and clinics, and workers of poultry farms and slaughter-house. Serum samples were examined by HI test for differentiate H<sub>5</sub>, H<sub>7</sub> and H<sub>9</sub> subtypes and sera with titers  $\geq 4$  (log<sub>2</sub>) were considered positive.

**RESULTS AND DISCUSSION:** 17.2% with  $21.14 \pm 10.59$  titer from poultry vaccinators and clinics sera and 12.8% with  $26.02 \pm 11.35$  titer from workers of poultry farms and slaughter-house sera were positive for H<sub>9</sub>N<sub>2</sub> influenza virus (HI titers  $\geq 1/20$ ). All tested sera were negative for H<sub>5</sub>N<sub>1</sub>, H<sub>5</sub>N<sub>2</sub>, H<sub>7</sub>N<sub>1</sub> and H<sub>7</sub>N<sub>7</sub> avian influenza viruses.

**CONCLUSION:** According to results of this study, different human population related to poultry industry were contacted with H<sub>9</sub>N<sub>2</sub> avian influenza virus that it should be critical during outbreaks of avian influenza subtypes posing a major public threat.

**Keywords:** Avian influenza viruses, Human population, HI test

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Seroprevalence of H<sub>5</sub> and H<sub>7</sub> Subtypes of avian influenza viruses in rural domestic poultry, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Influenza is one of the most important infectious viral diseases of birds, which causes huge economic losses to the poultry industry of countries every year. In recent years, H<sub>5</sub> and H<sub>7</sub> subtypes of influenza viruses (high pathogenic avian influenza) have been detected in Iran. Backyard poultry can cause spread these viruses to industrial poultry as reservoirs and vectors. Therefore, the aim of this study was to survey seroprevalence of H<sub>5</sub> and H<sub>7</sub> subtypes of avian influenza viruses in rural domestic poultry of Ardabil province, northwest of Iran.

### MATERIALS AND METHODS

This cross-sectional study was conducted from September to December of 2015. In this study, 943 blood serum samples were randomly collected from backyard poultry of 40 villages. The hemagglutination inhibition (HI) test was performed on the serum samples according to Iran Veterinary Organization (IVO) protocol to detect H<sub>5</sub> and H<sub>7</sub> subtypes of influenza virus.

### RESULTS AND DISCUSSION

All the examined sera were negative for H<sub>5</sub>N<sub>1</sub>, H<sub>5</sub>N<sub>2</sub>, H<sub>7</sub>N<sub>1</sub> and H<sub>7</sub>N<sub>7</sub> subtypes of influenza virus. The results of this study show no seroprevalence of H<sub>5</sub> and H<sub>7</sub> subtypes of influenza virus in rural domestic poultry of Ardabil province.

### CONCLUSION

Active surveillance must be carried out in rural domestic poultry.

**Keywords:** H<sub>5</sub> and H<sub>7</sub> viruses, rural domestic poultry, HI

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Running title: A narrative review on biofilm formation in the Mycobacterial genus and anti-mycobacterial agents

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

The development of biofilms is a characteristic pathogenic trait of mycobacteria. The objective of this systematic review is the formation of biofilm of the Mycobacterial Genus; the mechanism of biofilm formation, and Anti-mycobacterial biofilm agents.

##### MATERIALS AND METHODS

A systematic search was conducted to identify studies meeting our inclusion criteria in the Web of Science, PubMed, Embase, and Scopus electronic databases between 2000 and 2023. 150 papers were ultimately chosen for data extraction.

##### RESULTS AND DISCUSSION

The actions of tricarboxylic acid cycle (TCA) enzymes like 2-oxoglutarate dehydrogenase affect how *M. tuberculosis* biofilms develop. To control intracellular poly(P) homeostasis, *M. tuberculosis* expresses two polyphosphate kinases (PPK1, PPK2) and two exopolyphosphatases (PPX1, PPX2). PPK1 uses ATP hydrolysis to produce poly(P). Although PPK2 enzymes are capable of producing poly(P), *M. tuberculosis* PPK2 has nucleoside diphosphate kinase A-like activity and can catalyze poly(P) breakdown and ATP production 800-fold more quickly than poly(P) synthesis. *M. tuberculosis* cells that persist within biofilms exhibit greater antibiotic tolerance, just like other bacteria do

##### CONCLUSION

There have been new approaches created with potential antibiofilm compounds to increase therapy effectiveness. The proper therapy of patients with various NTM illnesses depends on a knowledge of biofilms, and the recent finding of *M. tuberculosis* biofilms has opened up a new area of study.

**Keywords:** Biofilm, Mycobacterium, *Mycobacterium tuberculosis*, non-tuberculous mycobacteria, anti-mycobacterial biofilm agents

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigation of frequency *stx1*, *stx2*, and *sta* genes in the isolates of *Escherichia coli* from Holstein Friesian calves, infected with coli bacillosis in Ilam province

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#### ABSTRACT

*Escherichia coli* is a natural part of the human intestinal flora of mammals and birds. *Escherichia coli* is generally an opportunistic pathogen that seeks to suppress the host immune system and the occurrence of the primary viral and microbial diseases of the respiratory tract occurs secondarily. *E. coli* in humans and mammals is often responsible for gastrointestinal infections. Generalized bacillosis occurs at different ages, but infection is more common in younger calves and is more common in children under three days of age.

Given that pathogenic *E. coli* species play an important role in food poisoning and livestock products can be a good sources that can cause pathogenicity in humans.

Measures should be taken to prevent human infection with this bacterium. This study aimed at molecular isolation of STX1, STX2 and STA genes in *E. coli* in calves with generalized bacillosis using culture; Multiple PCR was performed in Ilam city.

For this study, 100 *Escherichia coli* isolates were collected from calves with generalized bacillosis from farms in Ilam city. Samples in sterile containers were transferred to the microbiology laboratory in Ilam University as soon as possible.

Initial identification of isolates was performed using gram staining and growth in Mac conky agar medium. Additional biochemical tests were performed to confirm *E. coli* isolates. First, the isolates were cultured on EMB jellies and overnight at 37 °C. They were then incubated with lactose-positive colonies containing metallic polishes for final identification on TSI media MRVP, SIM and Simon citrate and urea and after biochemical approval, the desired isolates in TSB medium. According to the results obtained from 100 isolated isolates and according to molecular tests and polymerase chain reaction in total, 26% had the STX1 gene, 10% had the STX2 gene, and 33% had the STE gene.

**Keywords:** *E.coli*, Holstein friesian, *stx1*, *stx2*, and *sta* genes

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation, screening and molecular characterization of salt-tolerant bacteria from the rhizosphere of halophytic plants

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

Halophytic plants are evolved to grow in saline soils, but little is known about their associated microbial communities. This microbial community can be considered as a biofertilizer to improve plant nutrition and tolerance to abiotic stress. We isolated and screened the halotolerant bacteria from the rhizosphere zone of *Atriplex* sp., *Suaeda* sp., *Artemisia* sp., and *Bassia* sp. collected from pistachio orchards in Kerman Province.

##### MATERIALS AND METHODS

In this study, morphological characteristics and biochemical tests were used to differentiate all the isolates. Furthermore, the bacterial isolates were investigated for salt tolerance ranging from 5 to 15% and IAA production. The screened isolates were identified by sequencing of the V3-V4 region of the 16S rRNA gene. We evaluated the effectiveness of the PGPR strains in alleviating salinity stress on sweet corn in greenhouse condition.

##### RESULTS AND DISCUSSION

A total of 65 isolates have been characterized for their morphological and biochemical characteristics, of which 35 isolates were tolerant to 15% NaCl. Five isolates were characterized for the PGPR traits like indole acetic acid (IAA) production. The V3-V4 region of the 16S rRNA gene of the halotolerant PGPR isolates was successfully amplified using PCR, and approximately 460 bp of the amplified products were sequenced. The Phylogenetic analysis was performed based on the sequencing data.

##### CONCLUSION

In order to engineer biofertilizers with improved performance for sustainable agriculture, isolation and identification of new PGPR strains are necessary. Overall, the result showed that the five isolated bacterial strains have the potential to cope with the adverse effects of salinity stress.

**Keywords:** Biofertilizers, Soil salinity, Halophyte

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Fluoroquinolones-resistant investigation among *Acinetobacter baumannii* clinical isolates

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

In recent decades, *Acinetobacter baumannii* is considered a significant nosocomial pathogen on a global scale. Due to the excessive use of antibiotics, multi-drug resistant (MDR) *Acinetobacter baumannii* strains emerged, indicating high resistance to a broad spectrum of antibiotics. Fluoroquinolones such as ciprofloxacin and levofloxacin are viable choices in bacterial infection because of bacteriostatic activity and high oral bioavailability, but the extensive implementation of these antibiotics leads to increase fluoroquinolones-resistant bacteria. Thus, the determination of *Acinetobacter baumannii* sensitivity to Fluoroquinolones namely ciprofloxacin and levofloxacin seems necessary

### MATERIALS AND METHODS

One hundred isolates of *A. baumannii* were obtained between June and September of 2016 based on biochemical tests (growth on agar media, lack of fermentation of lactose, negative oxidase test, immobilization on the SIM environment, ALK / ALK pattern on TSI and non-production of pigment), and the molecular method (detection of blaOXA-51 gene). Antibiotic susceptibility tests for ciprofloxacin and levofloxacin were performed by E-test and broth dilution.

### RESULTS AND DISCUSSION

In this observational study, the blaOXA-51-like genes, which are considered unique to *A. baumannii* species, were present in all isolates (100/100). The distribution proportion of sample type was reported in Table 1. The E-test and broth dilution showed that 98% of isolates were resistant to ciprofloxacin and levofloxacin (98/100).

### CONCLUSION

In conclusion, the results indicated a substantial percentage of resistance to Ciprofloxacin and levofloxacin among samples of *Acinetobacter baumannii*. The connection between antibiotic usage and the development of antibiotic resistance is currently under investigation, however, it is a complicated matter with several factors to take into account. These factors include over usage of antibiotics, cross-transmission of resistance, transfer of resistance between different wards of a hospital, and community contributions to resistance. Genotyping investigations could provide wide insight into Ciprofloxacin and levofloxacin-resistance *Acinetobacter baumannii*.

**Keywords:** *Acinetobacter baumannii*, Fluoroquinolones-resistant, E-test, Broth dilution

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Isolation and molecular identification of *Zhihengliuella alba* from rhizosphere of *Bassia* sp.

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

The rhizosphere microbiome of halophyte plants harbors various groups of halotolerant plant growth-promoting rhizobacteria (HT-PGPR) that can increase plant growth and yield under salinity stress conditions. *Bassia* plants can be found in semideserts or dry steps, and, as halophytes, can exist in habitats characterized by high salinity. This study focused on the rhizosphere bacteria of *Bassia* sp., a halophytic plant collected from pistachio orchards.

##### MATERIALS AND METHODS

The *Bassia* plants in pistachio orchards were uprooted, and samples of soil adhering to the roots were collected into sterile bags for transport to the lab. To identify halotolerant PGPRs, the bacterial isolates were screened for halotolerance on NA supplemented with 0%, 5%, 10% and 15% NaCl at 28 °C. The halotolerant bacterial isolates were screened for IAA production. Molecular identification of the HT-PGPRs was performed by sequencing of V3-V4 region of the 16S rRNA gene.

##### RESULTS AND DISCUSSION

The pure culture of 14 rhizosphere bacterial isolates was prepared and subjected to biochemical tests. Most of them were able to grow at 15% salt concentration. Screening for IAA production by salkowsky's method showed positive results for only one strain. Based on the sequencing results of the V3-V4 region of the 16S rRNA gene, this isolate shared 99.50% similarity with *Zhihengliuella alba* strains deposited in Genbank.

##### CONCLUSION

The identification of HT-PGPRs from the rhizosphere of halophyte species in saline soils provides evidence that these halophiles can be used as inocula to promote the growth of salt-sensitive crops.

**Keywords:** PGPR, Soil salinity, Halophyte

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular Detection of Plasmid-mediated Gens in Colistin Resistant *Pseudomonas aeruginosa* Isolates in North of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa* is environmental bacterium with ability of making human infections like bacteremia, ventilator-associated pneumonia, urinary tract infections and skin soft tissue infections. The major problem is appearance of drug-resistant strains which makes the treatment process difficult in the face with this bacterium. The polymyxins, colistin (polymyxin E) and polymyxin B, are antimicrobial agents with broad-spectrum activity against Gram negative bacteria and it is considered as a last-resort antibiotic in treatment of *P. aeruginosa*. The emergence of colistin-resistant *Pseudomonas aeruginosa* is a serious worldwide concern and it became of a great threat for patients with severe infection with *P. aeruginosa*. Possible mechanisms for colistin resistance were studied by detection of *mcr-1* and *mcr-2* genes which are transferable plasmids. Therefore, the aim of this study was to detect plasmid-mediated *mcr-1* and *2* genes in colistin-resistant *P. aeruginosa* isolates in North of Iran.

### MATERIALS AND METHODS

A total of 190 clinical isolates of *P. aeruginosa* were collected from the two largest tertiary care hospitals in Babol, Iran. All bacterial isolates were phenotypically screened for colistin resistance using the colistin broth disk elution (CBDE) method. Moreover, *mcr-1-2* gene was detected through polymerase chain reaction (PCR).

### RESULTS AND DISCUSSION

Colistin resistance was found in 7.9% (15/190) of *P. aeruginosa* isolates. Also, 2.1% of isolates were intermediate resistant. Among 15 colistin resistant isolates, the *mcr-1* and *mcr-2* gene was not detected in any *P. aeruginosa*.

### CONCLUSION

This is the first report on the prevalence of the colistin-resistant *Pseudomonas aeruginosa* from humans in North of Iran which indicates an alarm for the manner and amount of antibiotics prescribed and used.

**Keywords:** *Pseudomonas aeruginosa*, colistin, *mcr-1*, *mcr-2*, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Case Report Support by Potato Dextrose Agar for Fungal and Bacterial Growth at 4°C

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#### ABSTRACT

Potato Dextrose Agar (PDA) has a potential to support the growth of bacteria and fungi and for isolation as well as enumeration of not only moulds but also yeasts. It is a cream to yellow colored medium that gets solidified in the petri dish when excess bacterial agar (3gm/100ml) is mixed prior to autoclaving. Its composition for 1 litre is as follows: potatoes, infusion-200 grams, dextrose (glucose)-20grams, agar-15 grams and pH at 25°C is 5.6±0.2.

The medium/refrigerator product for preservation is kept in the refrigerator at 4°C for preservation till the use. During the preservation, it is expected that the medium should be stable and contamination free. However, there is a shelf life to the PDA for storage in the refrigerator. After expiry of the refrigerator period, it gets dried rendering its uselessness for the further application. However, we reported unexpected observation on 1<sup>th</sup> October 2022. We made 200 ml PDA agar on 10<sup>th</sup> August 2022 using a ready to use bottle of a Himedia, autoclaved it at 121 °C for 1b pressure for 20 minutes as per the sterilization guidelines and poured in the 10 petri dishes in aseptic condition and then kept the medium for solidification for 30 minutes. After words, we placed the plates for refrigerator at 4°C. Then, we kept it under supervision and shocking, it was reported that the growth of black-white colored fungi (figure no1a) white colored fungi (figure 1c,1d) and pink colored bacteria (figure 2a, 2b) were observed with their luxuriant growths. The staining of fungi by lactophenol blue of black-white fungi (figure 1b) and white fungi (figure 1e) showed mycelium threads.

To add, we performed gram staining of the reported bacteria with pink colonies (figure 2c) and found that they were gram positive cocci. Interestingly, they also contaminated the green (figure 3) and white (figure 4) fungal culture preserved at 4°C for one month. In this case, we conclude that the PDA under study has been not protected at 4°C for 52 days from the psychrophilic growth raising the question on its efficiency and efficacy for long term preservation of microbial cultures suggesting an urgent need to search either alternate medium or updating the composition of it.

**Keywords: Potato Dextrose Agar Medium, Hi Media Product, Fungus, Bacteria**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibiotic Resistance Profile Among Uropathogenic *Escherichia coli* Isolated from Patients with Urinary tract infection in Hamedan, Iran

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Urinary tract infection (UTI) is one of the most frequent infectious diseases in human and can occur in all age groups. Uropathogenic *Escherichia coli* (UPEC) known as the main causative agent of this infection. Due to the different pattern of antibiotic resistance in *E. coli* isolated from different regions and different sources, it is necessary to study and check the antibiotic resistance profile of this bacterium. The aim of this study was to determine the antibiotic resistance pattern of UPEC isolated from urine samples of patients with UTI referred to hospitals in Hamedan city using phenotypic and genotypic methods.

### MATERIALS AND METHODS

The present study was conducted to determine the antibiotic resistance profile of 100 Uropathogenic *Escherichia coli* (UPEC) isolated from the urine samples of patients with UTI referred to hospitals in Hamadan, Iran. The antibacterial susceptibility testing of UPEC isolates was done using the Kirby-Bauer disk diffusion method based on the Clinical Laboratory Standard Institute guidelines (CLSI, 2021). In addition, the resistant isolates were examined by PCR for corresponding antibiotic resistance genes.

### RESULTS AND DISCUSSION

In this survey, 52 (52%) of UPEC isolates showed resistance to at least three antimicrobial families and were considered as the Multi-drug resistant (MDR). The highest resistance was against ampicillin (45%), tetracycline (40%), amoxicillin (51%) and Co-trimoxazole (55%) antibiotics. The most prevalent antibiotic resistance genes in UPEC strains were *blaTEM* (15.38%) and, *tetA*(15.38%) followed by *sulI*(13.46%) , *qnrA*(9.61%) , *aadA*(7.69%) , *dfrA1-like*(1.92%).

### CONCLUSION

Overall, to minimize the occurrence and dispersion of MDR in UPEC strains and other bacteria, application of antimicrobial stewardship in UTI treatment, precise hygienic control and, prevention strategies are suggested as an essential tool

**Keywords:** Antibiotic, Antibiotic Resistance gene, Multi-drug resistant (MDR), PCR, Urinary tract infection (UTI), Uropathogenic *Escherichia coli* (UPEC)

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation of *Helicobacter pylori* eradication potential of 18 *Hypericum perforatum* compounds on UreG protein by in silico method

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The *Helicobacter Pylori* is one of the most common pathogens for gastric infections in the worldwide. This bacteria is dependent on an acid resistance system to survive within gastric acid. This system mainly consists of Urease, which has the acid neutralization role. The Ni<sup>2+</sup> is considered as the cofactor of Urease. To provide it there is a protein complex, with UreG being the most influential part as it contains GTP binding site. The purpose of this research is to evaluate the inhibition potential of UreG by 18 selected *Hypericum Perforatum* compounds with the purpose to eliminate *Helicobacter Pylori* by gastric acid.

### MATERIALS AND METHODS

First, the structure of UreG was retrieved from the RCSB (PDB ID: 4HI0) and the active site was identified. Energy minimization of protein was performed by Swiss PDB viewer software with GROMOS96 force field. SDF files of 18 compounds of *Hypericum Perforatum* were downloaded from PubChem including Germacrene D, 3,8'-Biapigenin, Adhyperforin, Amentoflavone, Astilbin, Chlorogenic acid, Humulene, Hyperforin, Isoquercetin, Kaempferol, Khellin, Luteolin, Quercetin, Myricetin, Procyanidin B2, Pseudohypericin, Miquelianin, Rutin. Gridding and docking operations were ruined by PyRx software. Also, to compare these compounds with GTP as positive control, and Acetate, Phosphate, Benzene and Phenol as negative controls of UreG, docking operation was performed. The results were analyzed and finally, visual representation was prepared.

### RESULTS AND DISCUSSION

According to acquired results from 18 ligands, Amentoflavone with binding energy of -10.04 kcal/mol has the most appropriate affinity. In the ranking of 18 compounds, Humulene was the last with -5.6 kcal/mol binding energy. Also, the binding energy of GTP was -8.5 kcal/mol. Analysis of hydrogen bonds demonstrates that one bond forms between Amentoflavone and aspartate148 with 1.911 angstrom.

### CONCLUSION

Natural compounds have always attracted researchers due to high biocompatibility compared to synthetic compounds. Docking studies demonstrate pharmacodynamics effects of Amentoflavone, Rutin, Isoquercetin and 4 other compounds are better than GTP as substrate of UreG. Conformational analysis indicates that the active site was blocked properly. Pharmacokinetics analysis are implying the appropriateness of mentioned compounds in ADMET properties. According to the present study, we suggest to perform more simulations and experimental studies on Amentoflavone, Rutin and Isoquercetin.

**Keywords:** *Helicobacter pylori*, Docking, UreG, Amentoflavone

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of expression systems utilized in monoclonal antibody production

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Monoclonal antibodies (mAbs) are innovative and promising tools utilized in targeting the desired epitope of a specific antigen with highest affinity in order to enhance the functionality of immune system in response to potential immunogens. The aim of this review is to evaluate the proficiency of hosts utilized in mAb production.

### MATERIALS AND METHODS

To conduct this research, we studied 32 research articles regarding mAb manufacture on PubMed and Google Scholar.

### RESULTS AND DISCUSSION

Hybridoma technology is the primitive and favored method employed in production of mAbs. Selection of an appropriate host is contingent on various factors; existence of a homologous protein in immunized species, compatibility of the protein with human body and intensity of induced immune response are all vital factors in this process. BALB/c is the most frequent mammalian host used in production of mAbs due to the phylogenetic similarities to humans. Rabbits are alternative competent hosts utilized in mAb production due to offering superior characteristics such as 10x to 100x more affinity comparing to mice. Despite the commendable attempts to tackle the lack of myeloma cell line in this host with inducing viral transformation, genetic instability of cell line has led to limited success. Utilization of human hosts might seem to be the most convenient approach as they eliminate the necessity of molecular post-modifications and the products will be highly compatible. However, limited number of fusion partners and low efficiency of human cells unveil further challenges. Yeasts, insects and plants are substitute expression systems utilized in mAb manufacture. Phage display is an alternative and auspicious technique used for expression of desired mAb on surface protein of a bacteriophage as it provides a vast number of potent expression systems. Yet, folding problems of the bioproducts and limited expression capability of viruses deliver novel obstacles to resolve.

### CONCLUSION

MAbs acquire appreciable utility as well as apparent issues to be addressed. Selection of a well-qualified host in the production process plays a vital role in maximizing the efficiency of manufacture. Propitious outcomes of mAb applications in combination with additional therapeutics, encourage further studies in this regard.

**Keywords:** Monoclonal Antibody, Hybridoma, Phage Display, Expression System

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Can oral bacteria lead to memory loss?

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#### ABSTRACT

Dementia causes the decline of behavioral skills, functional ability and cognitive functions which affected language skill, memory, visual perception, problem solving, self-management and pay attention. Periodontitis, as chronic inflammation, is a risk factor in dementia and Alzheimer's disease (AD). Several factors disrupts oral homeostasis including genetic, nutrients, smoking, oxygen tension, age, diet, oral hygiene, vaccines, antimicrobials and biomaterials composition like implant and denture. Periodontal pathogen related with gastrointestinal diseases like appendicitis, as well as head and neck infections. Polymicrobial infections (e.g., *T. forsythia*, *T. denticola*, *F. nucleatum* and *P. gingivalis*) caused periodontal pockets, alveolar bone resorption and inflammatory responses. *P. gingivalis*, as gram-negative anaerobic bacterium, aggravate tau protein phosphorylation, amyloid deposition, neuroinflammation, cognitive impairment, and dementia. In carrying apoE4 allele subjects, *P. gingivalis*, Gram-negative anaerobic bacterium, could active microglia through tyrosine kinase binding protein (TYR-OBP) which expressed on myeloid cells-2 for suppression of immune system. Lipopolysaccharide of *P. gingivalis* caused cognitive dysfunction, learning impairment and memory loss through activation of the Toll- like receptors (TLR4) signalling pathway. In addition, gingipain (protease that produced by *P. gingivalis*) detected in AD brain patients which used as dementia biomarker. Gingipain load in AD brain suggested the relation between *P. gingivalis* and dementia or low number of teeth and cognitive dysfunction in apoE4 subjects. Gingipain inhibitors like COR388 and COR286 prevented the colonization of *P. gingivalis* in the brain, prevented the dementia and useful in treatment of aspiration pneumonia, rheumatoid arthritis, PerioD and AD.

**Keywords: Dementia; Porphyromonas gingivalis; Alzheimer's Disease; Inflammation**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Periodontitis, oral microbiota and Alzheimer's disease

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#### ABSTRACT

Periodontitis is an oral infection caused by destruction of periodontal tissue and inflammation due to microbial activity. Chronic periodontitis increases the risk of multiple tooth loss, systemic disease and cancer, renal and respiratory diseases, malignancies of the head and neck, intermittent pain, periodontal defects, masticatory dysfunction, and edentulous which affect patient quality of life, eating habits and self-esteem. The most important factors influencing periodontal disease include environmental (stress, tobacco smoking, and symbiotic relationships), acquired (poor oral hygiene practice, poor lifestyle, low socioeconomic status, carbohydrate diet, nutritional supplements, obesity, smoking, alcohol intake, drugs such as cyclosporine, nifedipine and phenytoin) and genetic factors (ANRIL, GLT6D1 and COX2). Chronic periodontitis occurs in elderly patients primarily associated with primarily dental plaque. Chronic periodontitis is treated surgically (surgical treatment of deep pockets and bony margins) and non-surgical approaches such as patient education, oral hygiene instruction, scaling and root planing (SRP), occlusal adjustment, antimicrobial agents, antibiotics, photodynamic therapy and use of probiotics. Periodontal treatment reduced pain, bleeding, pocket depth (PD), prevented bone destruction and decreased tooth mobility and tooth loss. *P. gingivalis*- Lipopolysaccharide caused low number of teeth, cognitive dysfunction, learning impairment and memory loss. *P. gingivalis*-LPS penetrates the brain and neurons to stimulate brain endothelial cells for releasing proinflammatory cytokines (IL-6 and CCL2) that causing memory and learning deficits, and exacerbate mild cognitive impairment to Alzheimer's disease (AD) progression. Therefore, there are correlation between periodontal pathogens, dementia, cognitive impairment, neurodegenerative disease, as well as the initial onset of AD and progression of AD.

**Keywords:** Aggressive Periodontitis; Alzheimer's Disease; Cognitive dysfunction; Microbiota

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Frequency of Quinolone Resistance Associated Genes in Clinical Isolates of *E. coli* Isolated from Urinary Tract Infections in Rasht

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Fluoroquinolones are a class of broad-spectrum antimicrobial agents which often used for the treatment of lower UTIs. We aimed to determine the frequency of quinolone resistance genes in *E. coli* isolated from urinary tract infection in Rasht city.

### MATERIALS AND METHODS

Resistance of 200 clinical isolates of *E. coli* to common fluoroquinolones and the MIC of ciprofloxacin were determined according to the clinical and laboratory standard institute guideline. Frequency of 5 plasmid mediated quinolone resistance genes *qnrA*, *qnrB*, *qnrS* were investigated by PCR.

### RESULTS AND DISCUSSION

According to phenotypic assays, 110 isolates (55%) were resistant to at least one quinolone compounds and 50 (25%) isolates showed high-level ciprofloxacin resistance. Among the PMQR tested genes, *qnrS* was the most common PMQR gene detected in this study ( $n = 84$ ), followed by *qnrB* ( $n = 56$ ), and *qnrA* ( $n = 17$ ).

### CONCLUSION

The potential for dissemination of these resistance genes poses a serious threat to the management of infections by these bacteria and compromised therapeutic options in resistance strains.

**Keywords:** Urinary tract infection, quinolone resistance, *E. coli*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating antibiotic resistance pattern and the presence of *sea* and *seb* genes in *Staphylococcus aureus* isolated from creamy pastry in Ilam

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#### ABSTRACT

The sweeter than a favorite human food, which has increased the consumption in many countries in recent decades. On the other hand, enterotoxins of *Staphylococcus aureus* are important factors in bacterial pathogenesis. Among them, enterotoxin A and B are known as the main cause of staphylococcal food poisoning in the world. Although the disease is mild and self-limiting and the mortality rate in this type of poisoning is low, but it is considered as one of the most important poisonings, in terms of health- economy, around the world. Recently, antibiotic resistance in bacteria has been considered a major public health problem. Food is an important factor in transmitting resistance to antibiotic agents. Residues of veterinary drugs in food can be carcinogenic, mutagenic, odd, allergenic, or toxic in humans. The presence of this bacterium or its toxins in food can be a potential risk to human health.

One hundred samples (cream bread and roulette) were taken from confectioneries in Ilam. Of the total 100 samples, 32 isolates (32%) were detected by biochemical tests as *Staphylococcus aureus* (23 isolates from roulette and 9 isolates from cream bread).

In this study resistance to tetracycline, erythromycin and oxacillin antibiotics in *Staphylococcus aureus* isolates from creamy pastry was checked by disk diffusion method. Also the presence of *sea* and *seb* genes by molecular method using PCR was screened.

In total of the isolates, 5 isolates (17.85%) was positive for *sea* gene, three isolates (10.71%) for *seb* gene and two isolates (7.14%) was positive for both genes. The study of antibiotic resistance of isolates also showed that on average 88% were resistant to tetracycline, 87% to erythromycin and 43% to oxacillin. The two antibiotics tetracycline and erythromycin had the highest resistance among isolates. The lowest resistance was related to gentamicin and imipenem. The results of the present study show a close relationship between erythromycin and tetracycline antibiotic resistance genes and *sea* gene.

**Keywords:** Antibiotic resistance, *Staphylococcus aureus*, Enterotoxin A and B, *femA*, *sea*, *mec A* and *seb* genes

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Postbiotics and evaluation of their effects in reducing blood lipids

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVE

The use of live bacteria as probiotics for humans may cause risks such as infection, inflammatory and local response. In people whose immune system is weakened, such as certain patients or patients who take immunosuppressive drugs such as MS organ transplantation for cancer patients, as well as genetic defect patients and pregnant mothers... and the possibility of transferring resistance genes. Antibiotic and disease can be expressed.

#### MATERIALS AND METHODS

This research is based on the study of related articles in Pubmed-ISI-Scopus and Google databases and studies on the effect of probiotics and Postbiotics.

#### RESULTS AND DISCUSSION

Postbiotics was evaluated on patients with high blood fat. Therefore, this research on the cellular components of probiotics is not necessary for probiotics to be healthy was used. Postbiotics are compounds with low molecular weight that are reproduced in the life cycle of probiotic bacteria in the intestine and their role is important in regulating growth, reproduction, cell-to-cell communication, and this molecule is a complex mixture of intracellular secretory substances and compounds. They are cell walls that produce various physiological effects, including various compounds such as fatty acid metabolites.

#### CONCLUSION

metabolites short chain (SCFAs) cellular fractions functional proteins extracellular polysaccharide teichoic acid mucopeptides. It is derived from peptidoglycan and cell wall structure post-biotic with effect on metabolic disorders through its anti-inflammatory and anti-hypo cholesterol effect in reducing blood lipids. and this study examines the effect of Postbiotics on reducing blood lipids in hypocholesterolemia

**Keywords:** Postbiotic, Probiotic, Hypercholesterolemia, Intestinal microbiota

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular detection of type III secretory toxins in MDR (Multi Drug Resistance) *Pseudomonas aeruginosa* isolates, from patients with burns and cystic fibrosis in Tehran hospitals

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### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa* is one of the main cause of infections associated with the intensive care unit (ICU). Several virulence factors are implicated in *Pseudomonas aeruginosa* colonization and invasion, making *Pseudomonas aeruginosa* infection's outcome worse. Type III secretion system (T3SS) effector proteins are among these virulence factors the purpose of this study, Molecular evaluation of type III secretory toxins in isolated MDR *Pseudomonas aeruginosa* from patients with burns and cystic fibrosis in Tehran hospitals.

#### MATERIALS AND METHODS

This cross-sectional study was conducted on 115 *Pseudomonas aeruginosa* bacteria samples from burn and cystic fibrosis patients.

All presumptive isolates were identified by standard microbiologic tests. Antimicrobial susceptibility test was carried out by disk diffusion method. The presence of virulence genes was determined by PCR method.

#### RESULTS AND DISCUSSION

In this study, 47 (40.9%) female samples and 68 (58.3%) male samples were isolated. 39 samples were from cystic fibrosis patients and 76 samples were from burn patients. The highest drug sensitivity was reported for the antibiotics colistin (98.3%) and ceftazidime, avibactam (91.3%), and the highest drug resistance was reported for the antibiotics gentamicin (92.2%) and meropenem (89.6%). The prevalence of genes *exoS* (93%), *exoT* (100%), *exo U* (82.6%) was.

#### CONCLUSION

Overall, the association of MDR and the presence of the specific virulence genes can be a predictive marker for the persistence of these isolates in the hospitals and subsequently a worse clinical condition for the affected patients.

**Keywords:** *Pseudomonas aeruginosa*, Antimicrobial resistance, type III secretion system

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The combination of vancomycin and floxacillin inhibits cell growth in methicillin-resistant *Staphylococcus aureus*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat regarding to nosocomial infections. Methicillin resistant (*mecA*), aminoglycoside resistant (*aacA*) and erythromycin resistant *ermA* are antibiotic resistant genes for MRSA. The combination of vancomycin with floxacillin can improve clinical outcomes. This study aimed to evaluate the frequency of antibiotic resistant genes and examine the combination effects of vancomycin and floxacillin against MRSA isolated from hospitalized patients in Gachsaran, Iran.

### MATERIALS AND METHODS

In this cross-sectional study of MRSA isolated and identified from patients with urinary tract infections, we set out to describe the frequency of antibiotic resistant genes, their antibacterial susceptibility patterns and combination effects of vancomycin and floxacillin according to standard guidelines.

### RESULTS AND DISCUSSION

Among the 90 clinical isolates of *S. aureus*, 24 isolates were identified as MRSA by standard laboratory methods. The frequency of antibiotic resistant genes, *mecA*, *aacA* and *ermA* were 17/2%, 86/2% and 48.3%, respectively. Based on the susceptibility results, we observed 93/1% susceptibility to vancomycin. We found the highest rates of resistance to ceftazidime, oxacillin and ampicillin, respectively. Vancomycin showed synergistic (68.97%), and additive (31.03%) interaction with floxacillin against clinical isolates of MRSA.

### CONCLUSION

Our studies may well confirm that *aacA* and *ermA* may influence the antibiotic resistant in MRSA. In addition, we identified a potentially therapeutically significant combination of the vancomycin and floxacillin in MRSA.

**Keywords:** Combination therapy; Floxacillin; MRSA; Vancomycin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Descriptive study of Pertussis among children admitted with diagnosis of Pertussis-like syndrome in Tabriz children Medical Center

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#### ABSTRACT

##### BACKGROUND AND ABJECTIVE

Whooping cough is a highly contagious acute bacterial respiratory tract infection which is caused by gram negative anaerobic coccobacillus known as Bordetella Pertussis. whooping cough is a disease with a global scope and can cause disease in all age groups, including children and adults. Pertussis is a major cause of mortality and morbidity in infancy and childhood. The infant mortality in developing countries can reach 4%. Based on studies the source of infectious agent in the newborn and infants is the bacteria in the respiratory tract of adults without typical symptoms whose illness is undiagnosed and untreated. The aim of this study was to evaluate the prevalence and epidemiology, laboratory and clinical symptoms of pertussis in hospitalized patients diagnosed with pertussis-like illness and determine the prevalence of cough in family members of the patients.

##### MATERIALS AND METHODS

In a descriptive cross-sectional study 182 children with clinical symptoms of cough diagnosed with pertussis-like illness for 26 months in Tabriz children hospital were studied. Based on national guideline for surveillance of pertussis their information was gathered and nasopharyngeal samples were sent for culture. Patient's epidemiological, clinical and laboratory information were collected and analyzed.

##### RESULTS AND DISCUSSION

182 patients admitted with suspected pertussis disease and nasopharyngeal samples were sent for culture. Among them 25 patients (13.7%) had positive culture and the diagnosis of pertussis was confirmed in such patients. Among patient's with positive cultures only one patient (4%) had received all 3 doses of DTP vaccine. The rest of the patients received fewer than 3 doses of the vaccine or didn't receive DTP vaccine. IN 10 patients (40%) there was a history of cough in the family members. Length of hospital stay was an average of 7.08 days.

##### CONCLUSION

Due to an outbreak of whooping cough in patients diagnosed with pertussis-like illness, especially in infants and children who have low immunity to disease increased awareness and more attention of pediatricians' residents and medical students about the disease is essential. Also, due to the fact that the 40% of patients have a family history of cough, the use of the booster dose of pertussis vaccine in adults to reduce the transmission of the disease appears to be necessary.

**Keywords: Pertussis-Epidemiology- Vaccination**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# New modified diagnosis method for detection Demodex mites in affected people with demodicosis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Demodex mites are the most common human ectoparasites. Demodex species can be found on all skin types across a broad geographical range. Demodex mites reside in the pilosebaceous units of the skin. Demodex mites feed on epithelial and glandular cells as well as sebum typically secreted by active pilosebaceous units. Demodicosis is the infestation of Demodex mites on the face, whereby a minimum of 5 mites/cm<sup>2</sup> exist and induce symptoms such as redness of the skin (erythema), telangiectasia, itching, heat, scaling, papules, pustules, and dermatitis, usually accompanied by a burning or pruritic sensation. Bacterial folliculitis, rosacea, seborrheic dermatitis and other common skin conditions have been linked to infestation by Demodex mites (human demodicosis). The diagnosis of demodicosis should be established by the visualization of Demodex mites in high numbers.

### MATERIALS AND METHODS

We used new method for observation Demodex mites precisely. Considering the diameter of the head of sampling tool is 2 mm, in order to sample an area of 1 square centimeter of the skin, it is necessary to sample the sebum of 5 areas of facial skin with a length of 10 mm. These five areas include 10 mm areas from the top of both eyebrows, 10 mm areas from the right and left side of the nose, and a 10 mm area from the border line of the forehead and five areas of different place on scalp. Finally, we have collected a diameter of 1 square centimeter or 100 square millimeters. After transfer of sebum samples to drop of oil on a laboratory slides, a drop for facial skin and scalp, separately; Then we put a cover slide on each drop. The preparation was examined under a light microscope (Olympus SZX16 microscope) 40× and 100× magnification. Multiple sections (about 5 on average) on every slide were examined. All forms of Demodex follicularum and Demodex brevis mites: adult, larvae and egg forms were counted as results. (A positive level of  $\geq 5/cm^2$  area was considered as a criterion for demodex positivity in patients).

### RESULTS AND DISCUSSION

In this study we have modified Current diagnostic method and improve the defects of previous methods such as skin surface biopsy (SSB), Dermoscopy and etc . This new modified method has the advantage of being noninvasive, inexpensive, accurate and rapid.

### CONCLUSION

We investigated a new sampling method for determining the presence mites and the severity of Demodex infestation, in both the patient and control groups. Methods commonly used to determine demodex mite densities have many defects such as being time consuming and require specific equipment and a trained observer. The results of our study revealed that our new method based on direct microscopic identification of Demodex mites is a more sensitive method for detecting Demodex mite than SSSB and Dermoscopy. Our sampling method used was not invasive and results are more precise, fast and inexpensive.

**Keywords: Demodex mite, Diagnosis method, Demodicosis, Microscopic identification**

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# Determination of the Colloidization potential of a native soil bacterium on different soils

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Soils include various materials such as simple chemical structures, salts, complex organic and inorganic compounds. The most active particles of soil are colloids. Mineral colloids are a variety of clays and organic colloids are humus. Colloidization of soil has many benefits to soil, including: increasing the soil's ability to maintain moisture, preventing soil erosion by wind and rain, and improving the soil structure for plant growth. The purpose of this study was to determine the colloidization potential of a native soil bacteria on different types of soil. This strain, which belongs to the species *Bacillus thuringiensis*, has been isolated and identified in previous research.

### MATERIALS AND METHODS

In order to evaluate the soil colloidization, microscopic and macroscopic properties of soil structure were studied. Colloidal compounds produced by this bacterium, which are long-chain hydrocarbon compounds, were identified by GC MASS. Effect of the amount of bacterial inoculum in the soil and UV radiation on different soils were investigated.

### RESULTS AND DISCUSSION

The results of these studies showed that the optimum temperature for the colloidal activity of this bacterium in different soils is 27-30 °C. This strain is a halophilic bacteria and can withstand up to 10% sodium chloride, it could also maintain its colloidal ability in salty soils. Studies have shown that by increasing the amount of bacterial inoculation into the soil, colloidization occurred in a shorter time. The colloidization of the soil remained at colloid state after a long time, approximately three months period. Also the results showed that colloidization was better in soils that were exposed to UV rays.

### CONCLUSION

The performance of this colloidal bacterium confirmed by electronic microscope and photos showed that this strain improves structure and cohesion of soils. It is believed that the function of this strain prevents soil erosion and helps to improve the environmental conditions.

**Keywords:** Soil bacteria, Soil colloid compounds, Soil structure, humus

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation and identification of a cellulose-producing bacterial strain with a special structure and applicability in the medical industry

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Microbial cellulose is structurally similar to plant cellulose but with special characteristics. These unique features have caused microbial cellulose to be considered in various industries including medicine. This study was conducted with the aim of isolating and identifying cellulose-producing bacteria from various fruit, vegetable, and vinegar samples.

### MATERIALS AND METHODS

Samples prepared from some vegetables and fruits, fruit juices, and kinds of vinegar were cultured in a Hestrin-Schramm medium, and after isolation and confirmation of the presence of cellulose-producing bacteria, pure bacterial cellulose was produced. The strain was identified by polymerase chain reaction (PCR) method and cellulose microstructures were analyzed by scanning electron microscope (SEM) and biochemical analyses.

### RESULTS AND DISCUSSION

Out of a total of 40 investigated samples, one sample of grape vinegar was considered. Because the bacterial strain in it had the highest amount of cellulose production. The results of the optimization of the production conditions showed that the maximum amount of bacterial cellulose in the synthetic Hestrin -Schramm culture medium, pH = 6 and temperature of 30 °C, after 10 days of incubation, is equivalent to 37.5 g/L. Studies have shown that by doubling the incubation time and continuously increasing the culture medium, the production of bacterial cellulose has increased. The results of PCR tests showed that the isolated bacteria is a strain of *Acetobacter pasteurianus*. Also, investigations of the produced bacterial cellulose revealed that this cellulose has ribbon-shaped fibers, a porous structure, and pores in nanometer dimensions; without lignin, pectin, and hemicellulose; It has an extraordinary molecular structure and has very high characteristics of polymerization and liquid absorption capacity, which makes it a superior option to plant cellulose in many applications.

### CONCLUSION

According to the results of this research and the production of cellulose with special and desirable quality, this bacterium can be introduced as a new and native source of cellulose production and can be used in various industries in the field of medicine and biotechnology.

**Keywords:** Cellulose, Bacterial Cellulose, *Acetobacteriaceae*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Urinary tract infection by *Corynebacterium urealyticum*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVE

*Corynebacterium urealyticum* is a gram positive, slow growing, urease positive and microorganism with a diphtheroid morphology. This organism has long been recognized as a uropathogen because of its urease activity, which play an important role in the pathogenesis of UTIs, resulting in the alkalization of urine, further leading to encrusted cystitis or struvite stone formation. The risk factors constitute the elderly age group, immunosuppression, urological procedure, prior broad spectrum antibiotic therapy. *C. urealyticum* being a known multi drug resistant organism. Its treatment with the right antibiotic becomes significant, as failure in therapy leads to persistent infections. We report here case of *C. urealyticum* which caused uncomplicated UTI in a young patient with cleft lip case report.

#### MATERIALS AND METHODS

A 7 years old child with pre-natal conditions ultra red in near clinical laboratory. These patients suffer from dysuria and frequency and urgency of urination. The organism grows on Blood agar as pinpoint colonies after 48 hours of incubation at 35-37° C. Colonies are whitish, opaque, smooth, convex, circular and non hemolytic. This bacteria is strongly urease positive. Antimicrobial susceptibility showed susceptibility to all  $\beta$ -lactams and was sensitive to vancomycin.

#### RESULTS AND DISCUSSION

Growth in pure culture or as predominant flora, especially if seen with polymorphonuclear cells in gram stained samples is of great value. Most *C. urealyticum* isolates are missed in routine urine culture because the organism does not grow after overnight incubation. For diagnosing human infections caused by *C. urealyticum* strong collaboration between microbiologist and infectious diseases or urologist is necessary. The prognosis for patients with infection caused by *C. urealyticum* appears to be reasonably good.

**Keywords:** *Corynebacterium Urealyticum*, Urinary Tract Infection, Struvite Stone

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### *Aerococcus Urinae* In Urinary Tract Infection

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

There are three described species of the genus *Aerococcus*: *A. Viridans*, *A. Urinae* and *A. Christensenii*. *A. Christensenii* has not been reported as a human pathogen. *A. Urinae* indicate that it is associated with UTI, Bacteremia and Endocarditis. Most patients infected with *A. Urinae* are elderly males with predisposing conditions who present initially with UTI. We report one case of urinary tract infection caused by *A. Urinae* and characterize this isolate by morphology, biochemical testing and Antibiotic susceptibilities.

#### MATERIALS AND METHODS

A 42 years old male with history of diabetes mellitus presented at the Noor clinical laboratory with several days of dysuria, increased urinary frequency and nocturia. Urin analysis revealed many WBC/ HPF, many bacteria and negative result for nitrites. The urine culture grew  $> 10^5$  cfu/ml of *A. Urinae* the only isolates.

#### RESULTS AND DISCUSSION

The laboratory Diagnosis of *A. Urinae* can be difficult. The presumptive identification of aerobic gram positive, alpha- hemolytic cocci and the decision on whether to more fully identify the isolates based on gram stain, colony appearance and catalase reaction. Because *A. Urinae* is catalase negative, it could be mistaken for alpha- hemolytic streptococci or enterococci that are more common urine isolates. A gram stain should be carefully examined for the characteristic arrangement in clusters and tetrads to rule out streptococci. The most important routine tests are hydrolysis of hippurate, PYR and Antibiotic susceptibility test. This is important to consider *A. Urinae* as a potential pathogen, because when *A. Urinae* was not recognized or correct antibiotic treatment was delayed simple UTI progressed to systemic infection including endocarditis and sepsis.

**Keywords:** *Aerococcus Urinae*, Urinary Tract Infection

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The microorganism's role in the production of biogas and compost from anaerobic digestion of municipal solid waste

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

With increasing the urban population, management of municipal solid waste (MSW) is an environmental problem in worldwide that disturb human and animal health in most cities of developing countries. Anaerobic digestion is a very complex biochemical process that requires a large number of bacterial species in a consortium to hydrolyze organic matter into compost and produce biogas. The aim of this study was to review on studies about efficiency of producing biogas and compost from MSW.

### MATERIALS AND METHODS

Articles related to the subject were searched in Scopus and Science Direct databases and articles that evaluated the ability of biogas production from MSW.

### RESULTS AND DISCUSSION

Each anaerobic digester creates its own microbiome. At present, most of the species and therefore the ecological functions of the biogas microbiome are unknown. Most of these species are strict anaerobes such as *Clostridia*, *Bacteriocides* and *Bifidobacteria*, but facultative anaerobes such as *Streptococcus* and *Enterobacteriaceae* can also be present. Hydrogen-producing estogenic bacteria include bacteria such as *Acetobacterium woodii* and *Clostridium aticum*. Methanogenic bacteria produce methane from acetate, hydrogen and carbon dioxide. Biologically produced methane is an important alternative fuel source. Nutrients, humidity, particle size, organic loading rate (OLR), solid retention time, sulfate reduction, denitrification and ammonium concentration are effective factors in the growth and activity of biogas producing microorganisms. In addition to, compost production with the anaerobic digestion method is created in much less time than the aerobic method, which is equal to it in terms of quality and microbial load.

### CONCLUSION

Anaerobic digestion processes can provide an efficient management strategy for exploiting the organic fraction of municipal solid waste.

**Keywords:** Anaerobic digestion, Biogas, Organic fraction of municipal solid waste (OFMSW)

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Plasmid-mediated ciprofloxacin-resistance genes in *Pseudomonas aeruginosa* isolated from hospitalized patients in Zabol, Iran

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#### Abstract

#### BACKGROUND AND OBJECTIVES

Plasmid-dependent resistance to ciprofloxacin is increasingly spreading among *Pseudomonas aeruginosa* isolates worldwide. In this study, the evaluation of antibiotic resistance to ciprofloxacin and the presence of *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA* and *aac (6')-Ib-cr* genes in *Pseudomonas aeruginosa* isolated from hospitalized patients in Zabol was investigated.

#### MATERIALS AND METHOD

A total of 103 clinical isolates of *Pseudomonas aeruginosa* were collected from hospitalized patients. The bacterial isolates were identified through standard laboratory protocols. Antimicrobial susceptibility was determined by the standard disk diffusion method. The presence of *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, and *aac (6')-Ib-cr* genes was investigated by the polymerase chain reaction (PCR) method and sequencing.

#### RESULTS AND DISCUSSION

Overall, 37 (35.92%) isolates were non-susceptible to ciprofloxacin. The prevalence rates of *qnr* genes were as follows: *qnrA* (13.51%), *qnrB* (27.02%), *qnrS* (54.05%) and *aac (6')-Ib-cr* (83.78%). The *qnrC*, *qnrD*, and *qepA*, genes were not recognized in any isolates.

#### CONCLUSION:

The expansion of ciprofloxacin-resistant plasmid genes plays an important role in the prevalence of *Pseudomonas aeruginosa* ciprofloxacin-resistant strains.

**Keywords:** Antimicrobial resistance, *qnr* genes, *Pseudomonas aeruginosa*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Fabrication and evaluation of the antibacterial effects of nano-graphene films containing vancomycin compared to vancomycin

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Bacterial resistance is one of the significant medical problems over the past decades. A large number of diseases caused by bacterial agents and the development of multidrug resistance in genes have raised the need to produce new drug molecules or novel drug delivery systems for better treat diseases. Novel drug delivery systems emerged in which inorganic compounds of metal oxide nanoparticles such as silver, gold, copper, titanium, and zinc have shown remarkable antibacterial activity. Adding nanoparticles to a polymeric material increases the likelihood that they will affect bacterial activity. As mentioned above, this study aimed to fabricate nanographene films containing vancomycin and investigate their antibacterial effects.

### MATERIALS AND METHODS

Gram-positive *Staphylococcus aureus* bacteria were identified by mannitol salt agar culture medium and coagulase test. FT-IR, SEM, thermal gravimetric analysis (TGA), and differential thermal analysis (DTA) were used to detect the morphology of the film and to identify the constituent elements. Also, to investigate the antibacterial effects of the films, the disk diffusion method was used. Based on the inhibition zone, the growth inhibition was calculated and compared to the control.

### RESULTS AND DISCUSSION

Examining the results of the inhibition zone, it was observed that the graphene oxide disk alone does not have significant antibiotic properties. Strong antibacterial effects were observed in vancomycin discs. Graphene oxide-vancomycin complex disks also showed significant antibiotic effects on various strains of *S. aureus*.

### CONCLUSION

It was observed that the formation of graphene oxide-vancomycin complex in some strains of *S. aureus* has even more controlled antibiotic effects compared to vancomycin alone.

**Keywords: Vancomycin, Nanoparticles, Graphene Oxide, Disc Diffusion.**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### A comparative analysis of codon usage and CAI in hsv-1 and hsv-2

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Glycoprotein D in herpes simplex viruses is involved in binding to the receptor of host cells and the process of entering the cell.

#### MATERIALS AND METHODS

In this study, the glycoprotein D gene was investigated in two human herpes simplex viruses type 1 and 2 using the <http://genomes.urv.cat/CAIcal> server. First, by extracting the sequence of gD genes from the NCBI database, the coding sequence (CDS) of these genes was identified and selected, then these two sequences were used as raw data for the various sections of the mentioned server. The standard codon usage table and human codon usage were used as reference sets. Using these two sequences, different components such as sequence length, nucleotide composition, codon usage, and Relative Synonymous Codon Usage (RSCU) were calculated.

#### RESULTS AND DISCUSSION

These investigations showed that according to the sequence length and similar G+C percentage, in each of the viruses, some codons of amino acids may be used more, and some codons may never be used. codon UUA (leucine) and codon GGU (glycine) in HSV-1 and codon UCA (proline), codon CCU (cysteine) and codon AGG (arginine) in HSV-2, as well as codon AGA (arginine) in both viruses to make Glycoprotein D have never been used and has 0% codon usage. Among the translation termination codons in both types of viruses, only the UAG codon is used. In another option of the server, which was related to the calculation of codon adaptation index (CAI), two parameters CAI and expected CAI were calculated. eCAI provides a direct threshold value that makes it possible to discern whether differences in the CAI value are statistically significant or merely artifacts arising from internal biases in the G+C composition and/or the amino acid composition of the query sequences. The CAI of the sequences is equal to 0.792 and 0.757 for HSV1- and HSV-2, respectively. And the expected CAI (eCAI) for these sequences is 0.804 at the 95% confidence level and 0.836 at the 99% confidence level.

#### CONCLUSION

This shows that the CAI of both sequences is not significantly higher than the expected CAI at both confidence levels due to their nucleotide and amino acid composition.

**Keywords:** Herpes simplex viruses, gD glycoprotein, CAIcal server, Codon usage

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The Synergistic Effects of Homogenized Coumarin and Bacterial Nanocellulose on Skin Wound Healing in a Rat Model

Mohamad Bagher Noori<sup>1</sup>, Fatemeh Darestani<sup>1</sup>, Hadi Cheraghi<sup>1\*</sup>, Dr Ali Ghashghaei<sup>1\*</sup>

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

The process of wound healing, composed of coagulation, inflammation, tissue regeneration, and structure rejuvenation, is a highly complex biochemical process. In order to reinforce this process, our study has investigated the medicinal properties of a combination of coumarin and bacterial nanocellulose for wound healing.

### MATERIALS AND METHODS

Using full-thickness excisional wounds inflicted on the dorsal area of adult female Sprague Dawley rats, we established five treatment groups: one with sterile gauze (control), one with basal cream ointment, one with 2% coumarin cream, one combining 2% coumarin and nanocellulose, and another with nanocellulose alone. We assessed the wound area regularly and conducted histological analyses on post-wound days 7, 14, and 21.

### RESULTS AND DISCUSSION

The findings exhibited significant advancements in wound closure rates, hydroxyproline content, collagen deposition, and histopathological features, particularly on day 7, for the coumarin and coumarin + nanocellulose groups compared to the control group and other treatments. In these groups, there was also a marked improvement in the oxidative stress index, an indicator of inflammation and oxidative stress.

### CONCLUSION

This research indicates the strong potential for coumarin alone and in combination with bacterial nanocellulose to improve the healing of skin wounds in a rat model. Potentially promising treatment options for wound healing in preclinical and animal and human models of various types of wounds have been identified based on improvements in wound closure, Collagen synthesis, and reducing oxidative stress. In future animal and human trial studies to assess their safety and effectiveness, cellular and molecular mechanisms behind these effects should be explored.

**Keywords:** Wound Healing, Coumarin, Nanocellulose, Oxidative Stress, Collagen

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Identification of *Candida* species isolated from oral infection of head and neck cancer patients by PCR RFLP method

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Candida* species are the most important cause of nosocomial fungal infections. Oropharyngeal candidiasis is a common disorder in patients with head and neck cancer that occurs during radiotherapy and begins with a post-colonization phase of the oral mucosa. Due to the importance of detecting *Candida* species, this study was conducted in order to identify the species by RFLP molecular method to recognize this yeast.

### MATERIALS AND METHODS

Oral swab samples were all cultured on Sabouraud Dextrose Agar medium for 48 h at 28 °C and then sub-cultured on a chromogenic medium. Final identification was confirmed by PCR RFLP method. All polymerase chain reaction (PCR) products underwent digestion by restriction enzyme MspI. The digested products were electrophoresed on agarose gel 1.7%. Each isolate was identified by comparing the obtained band patterns for *Candida* species.

### RESULTS AND DISCUSSION

Our results showed that the most frequently *Candida* species from a total of 23 isolates was *C. albicans* (65.21%) followed by *C. tropicalis* (26.08%) and *C. glabrata* (8.69%).

### CONCLUSION

Among the 23 isolates isolated from patients with oropharyngeal candidiasis in this study, based on the molecular method, *Candida albicans* was the most prevalent. *Candida tropicalis* ranks second and *Candida glabrata* has the lowest prevalence.

**Keywords:** Oral candidiasis, Identification, Head and neck cancer, PCR RFLP

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of the effect of curcumin-ciprofloxacin encapsulated chitosan nanoparticles on *Pseudomonas aeruginosa* motility

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*P. aeruginosa* is a gram-negative opportunistic bacterial pathogen. The ability of *P. aeruginosa* to cause chronic infection is attributed to several factors such as biofilm formation, intrinsic resistance to antibiotics, and QS-dependent virulence traits. Curcumin is a polyphenol compound with anti-tumor, antioxidant, anti-inflammatory, antimicrobial, and anti-biofilm properties. Therefore, the current work was performed to evaluate the effect of curcumin-ciprofloxacin encapsulated chitosan nanoparticles (Cur-Cip chitosan NPs) on *P. aeruginosa* motility.

### MATERIALS AND METHODS

To evaluate the swarming motility, a nutrient agar medium containing 5% agar and Cur-Cip chitosan NPs (640 mg/mL) was prepared in 6 cm Petri dishes. Then, 2  $\mu$ L of bacterial suspension was placed on the surface of the medium. After incubation, the swarming zone diameter was measured. The effect of the NPs on twitching motility in a nutrient agar medium (1.5% agar) containing Cur-Cip chitosan NPs was evaluated. The bacterial suspension was inoculated at the bottom of the plates and after incubation, the medium was removed and the plates were stained with crystal violet and the twitching motility zone was measured. To evaluate bacterial swimming, nutrient broth containing 0.3% agar and the NPs was prepared in Petri dishes and the bacterial suspension was inoculated in the center of the agar layer. After incubation, the swimming zone was measured.

### RESULTS AND DISCUSSION

Cur-Cip chitosan NPs significantly reduced the swarming, swimming, and twitching motility of *P. aeruginosa*. The swimming and swarming motility zones in the presence of the NPs reduced by 65% and 45%, respectively. Also, bacterial twitching was almost completely inhibited in nanoparticles-treated bacteria.

### CONCLUSION

Cur-Cip chitosan NPs exhibited considerable inhibitory effects on the motility of *P. aeruginosa* strains. Due to the role of bacterial motility in the pathogenesis of *P. aeruginosa*, Cur-Cip chitosan NPs can be considered a potent anti-virulence compound against pathogenic *P. aeruginosa* strains.

**Keywords:** Curcumin, Chitosan, Ciprofloxacin, Twitching.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of Antibiotic Resistance in the *Mannhemia hemolytica* Isolates and Their Association with Class I and II Integrons

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Mannhemia hemolytica* is a gram-negative, coccobacillus. As a secondary pathogen, it plays an important role in the progression of severe pleuropneumonia in cattle, sheep and goats. Due to the excessive use of antibiotics for the treatment of pleuropneumonia, *M. hemolytica* has become resistant to a large number of antibiotics. Resistance genes have been associated with small plasmids and transposons. In this research, we investigated the resistance to common antibiotics used against *M. hemolytica* infection and class I and II integrons and their relationship with antibiotic resistance.

### MATERIALS AND METHODS

35 *M. hemolytica* isolates from the lungs of sheep that stored in the archives of bacteriology department of the Shiraz Veterinary Faculty were used. After the phenotypic confirmation of the isolates with biochemical tests (catalase, oxidase and hemolysis) resistance to streptomycin, chloramphenicol, penicillin, and gentamicin was evaluated by disk diffusion method. Then, the presence of class I and II integrons were checked by PCR.

### RESULTS AND DISCUSSION

According to these results, the highest resistances were observed to the gentamicin, erythromycin and streptomycin and the lowest resistances of the isolates were shown to tylosin and amoxicillin. The PCR results for the presence of class I and II integrons showed that among the 35 isolates, 24 isolates had class I integron and 30 isolates had class II integron. The statistical analysis of the results showed that there is no significant relationship between the presence of class I integrons and the increase in relative resistance to the studied antibiotic ( $P>0/05$ ). Conversely, the frequency of resistance to amoxicillin was significantly higher in strains lacking class II integron ( $P=0/017$ ). No significant correlation was observed between the simultaneous presence of class I and II integrons and the frequency of resistance to any of the studied antibiotics.

### CONCLUSION

According to the different researches class I and II integrons play a key role in the antibiotic resistance of *M. hemolytica* isolates. Also, with the passage of time and genetic exchanges between *M. hemolytica* isolates, antibiotic resistance genes are transferred and antibiotic resistance increases. There is a need for further study and strict regulations to maintain the effectiveness of drugs and prevent the spread of antibiotic resistance.

**Keywords:** Resistance, *Mannhemia hemolytica*, Pleuropneumonia, Integron, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Molecular detection of plasmid mediated colistin resistance genes (*mcr*) in multiple drug resistant *Escherichia coli* isolated from urinary tract infection in humans

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Antibiotic resistance represents a real health emergency worldwide, mostly due to the lack of new antibiotics active against multi drug resistant gram negative bacteria. According to the global epidemiological situation, in many infections, including urinary tract infections, some antibiotics such as quinolones and sulfonamides are less effective than in the past due to the high level of resistance. Colistin is recommended to use as the last option for the treatment of infection caused by gram-negative bacteria which is resistant to several drugs by the World Health Organization. The emergence of colistin resistant (*mcr*) plasmid gene in China in 2015 and its spread in different countries is the main public health concern in recent years. The purpose of this study is to investigate the presence of *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* in *Escherichia coli* isolates causing urinary tract infections.

### MATERIALS AND METHODS

In this study, seventy *Escherichia coli* isolates that cause urinary tract infections with multiple resistance and 30 resistant urinary *Escherichia coli* isolates from Ghaem hospital in Mashhad were examined. Using multiplex PCR, *mcr-1*, *mcr-2*, *mcr-3* and *mcr-4*, *mcr-5* genes were analyzed together.

### RESULTS AND DISCUSSION

There are more than 9 different types of *mcr* gene, and only five genes were examined in this study and there was no evidence of the presence of five resistance genes in this study. It is possible that the resistance to colistin is due to the presence of other variants and the presence of chromosomal resistance genes. Another interesting discussion is related to the high use of colistin in the pig industry. According to surveys, the countries that produce and consume the pork also have the most reports of the *mcr* genes. As a hypothesis, pigs can be assumed as the source of *mcr* genes expansion.

### CONCLUSION

This study serves as a clue for further studies to investigate different variants of the *mcr* genes. It can also be concluded that probably other mechanisms besides the *mcr* plasmid genes cause resistance to colistin.

**Keywords:** Resistance, Urinary tract infection, *Escherichia coli*, Colistin, UPEC, *mcr*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# High Prevalence of Extended Spectrum $\beta$ -lactamases producing *Escherichia coli* in Patients with Urinary tract infection in Hamedan, Iran

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Urinary tract infection (UTI) is one of the most common infections in humans which probably affects one-half of all people during their lifetimes. Uropathogenic *Escherichia coli* (UPEC), one of the members of the extra-intestinal pathogenic *E. coli* (ExPEC), is the main etiologic agent of UTI. The excessive and inappropriate use of antimicrobial agents specially  $\beta$ -lactam antibiotics for treatment of UTI infections has recently led to the emergence of multiple drug resistance (MDR) and increasing in Extended Spectrum  $\beta$ -lactamases (ESBL) producing *E. coli*, worldwide. The present study was conducted to investigate the presence of ESBLs among the UPECs isolated from patients with UTI referred to hospitals in Hamedan city using phenotypic and molecular methods.

### MATERIALS AND METHODS

A total of 100 UPEC isolates, were used in this study. These strains were isolated from the patients with UTI referred to hospitals in Hamedan city, Hamedan, Iran. Bacterial isolation and identification was done using conventional microbiology methods and standard biochemical tests, additional identification was made using molecular methods. The UPEC isolates were screened for ESBLs production by the double-disk synergy test using Ceftazidime (30  $\mu$ g) and Cefotaxime (30  $\mu$ g) disks and confirmed by combined disk diffusion test using Clavulanic acid according to the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI, 2021). The presence of four  $\beta$ -lactamases gene including *bla<sub>TEM</sub>*, *bla<sub>SHV</sub>*, *bla<sub>OXA</sub>*, and *bla<sub>CTX-M</sub>* among ESBLs producer UPEC isolates ( $n=40$ ) was investigated using PCR

### RESULTS AND DISCUSSION

From a total of 100 UPEC isolates, 40 (40%) isolates were phenotypically identified as ESBLs producers. Among 40 ESBLs producers UPEC isolate the most prevalent  $\beta$ -lactamases gene was *bla<sub>CTX-M</sub>* 19/40 (47.5%) followed by *bla<sub>TEM</sub>* 22.5% (9/40) and *bla<sub>OXA</sub>* 20 % (8/40). The *bla<sub>SHV</sub>* gene was not found in any tested isolates.

### CONCLUSION

Due to relatively high prevalence of ESBLs producer UPEC isolates in patients with UTI and incidence of  $\beta$ -lactamases gene among these isolate, applying precise antibiotic stewardship, continuous surveillance in order to use appropriate antibiotics, and the control of UTI infections seem necessary to decrease ESBLs producer UPEC.

**Keywords:** Extended Spectrum  $\beta$ -lactamases (ESBL), Uropathogenic *Escherichia coli* (UPEC), Urinary tract infection (UTI),  $\beta$ -lactamases gene, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Combining antibacterial antibiotics with artemisinin for controlling resistant pathogens

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nearly 80 years of antibiotic use shows that resistant pathogens emerge a few years after the introduction of an antibiotic. Our study focuses on innovative therapeutic strategies that are currently important against multi-drug-resistant microorganisms, such as plant-derived products.

### MATERIALS AND METHODS

This research is a review study that examines reputable English-language articles and electronic databases and libraries such as Web of Science, ProQuest, PubMed, Scopus, and the Google Scholar search engine using keywords like antibiotic, antimicrobial resistance, and artemisinin.

### RESULTS AND DISCUSSION

Antimicrobial resistance is the ability of a microorganism to resist various antimicrobial actions. In this type of resistance, microbes can resist drugs that were once successful against them. The current research needs to look for biomolecules that are themselves antimicrobial and enhance the power of antimicrobials. The phytochemicals of medicinal plants have great potential for development as biological enhancers of antibiotics. Among plants, the Asteraceae family includes a large number of genera, with *Artemisia* being one of the largest genera worldwide.

*Artemisia annua L.*, one of the most recognized species of this genus, contains the compound artemisinin present in glandular trichomes on the surface of leaves and flowers, which has many anti-protozoal activities.

### CONCLUSION

Based on its antimicrobial potential, *A. annua* can be used to create a stronger antibiotic formulation for treating drug-resistant bacterial and fungal pathogens.

**Keywords:** Antibiotic, Antimicrobial resistance, *Artemisia annua*, Artemisinin.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Phenotypic and molecular investigation of virulence factors in *Pseudomonas aeruginosa* clinical isolates in Bushehr, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa* is an opportunistic pathogen known as one of the leading causes of nosocomial infections. The pathogenesis of *P. aeruginosa* includes several virulence factors that enable the bacterium to adapt to a wide range of environments. This study aims to the phenotypic and molecular investigation of virulence factors in *P. aeruginosa* clinical isolates in Bushehr, Iran.

### MATERIALS AND METHODS

One hundred and three *P. aeruginosa* clinical isolates were collected from three hospitals in Bushehr, Iran between October 2017 and June 2019. The isolates were examined for the production of lipase, protease, lecithinase, gelatinase, hemolysin, pigment, DNase, and biofilm. The presence of *exoT*, *exoA*, *exoY*, *exoS*, *exoU*, *lasB*, *aprA*, *algD*, *plcH*, and *nanI* genes were investigated by PCR and sequencing.

### RESULTS AND DISCUSSION

One hundred and one (98%) isolates produced protease, lecithinase, hemolysin, and pigment. Ninety six (93.2%) isolates were gelatinase producers, and 41 (39.8%) isolates were lipase producers. None of the isolates showed DNase activity. Out of the 103 isolates, 101 (98%) were biofilm producers, of which 69 (67%) isolates were strong biofilm producers, 16 (15.5%) isolates were moderate biofilm producers, and also 16 (15.5%) isolates were weak biofilm producers. Two (2%) isolates were identified as non-biofilm producers. *algD*, *lasB*, *aprA*, *plcH*, *exoA*, and *exoY* genes were found in 102 (99%), 101 (98%), 101 (98%), 99 (96.1%), 96 (93.2%), and 93 (90.3%) of the isolates, respectively. The frequency of *exoT*, *exoU*, *exoS*, and *nanI* genes was 75.7%, 62.1%, 38.8%, and 17.5% respectively. The results of our research revealed that the frequency of the isolates producing protease, lecithinase, gelatinase, hemolysin, pigment, and biofilm is high in Bushehr. Most isolates produced strong biofilms. The ability to form biofilm can be related to *algD* gene.

### CONCLUSION

The high prevalence of strong biofilm-producing isolates and related gene in our region emphasizes more attention to overcoming antimicrobial resistance

**Keywords:** *Pseudomonas aeruginosa*, virulence, biofilm, *exoA*, *exoU*, *algD*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Potential Antimicrobial properties of coffee waste against index bacterial strains

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Coffee is a very popular beverage and one of the most traded commodities in the world. The process of making coffee starts with harvesting the coffee fruit, and after undergoing some changes, it becomes a consumable product. During industrial coffee production, a significant amount of by-products such as fruit skins, fruit pulps, and coffee silver skin (also known as coffee waste) are generated, and most of them are discarded. In this study, we investigated the antimicrobial properties of Arabica and Robusta coffee waste.

#### MATERIALS AND METHODS

To begin the study, two types of coffee waste (Arabica and Robusta) were packed and transfer to the laboratory. First, The coffee waste extracted manually by using 70% alcohol for five minutes. Afterwards, secondary extraction was done by 4000 rpm centrifuge for 30 minutes, and finally, the solution filtered with a 0.45micron filter.

The MIC (Minimum Inhibitory Concentration) and MBC (Minimum bactericidal concentration) methods were conducted using two types of coffee waste extracts on four bacteria *E. coli*, *S. aureus*, *S. flexneri*, and MRSA (Methicillin Resistance *Staphylococcus aureus*).

#### RESULTS AND DISCUSSION

MIC test for *E. coli*, *S. aureus*, *S. flexneri*, and MRSA with Arabica coffee waste were 0/025 g/ml, 0/0125 g/ml, 0/025 g/ml, and 0/025 g/ml, respectively. Additionally, the MBC results showed the compactness 0/025 g/ml of Arabica coffee wastes. Furthermore, results of the MIC test for Robusta coffee waste were 0/05g/ml, 0/025 g/ml, 0/05g/ml, and 0/025 g/ml, respectively; also, the MBC test results illustrated the compactness 0.05 g/ml for *E. coli* and *S. flexneri* and 0.025 g/ml for *S. aureus* and MRSA of Robusta coffee waste. Results showed the coffee extracts of Arabica and Robusta had antimicrobial properties and demonstrated these coffees waste had an inhibitory effect on the growth of this strain of bacteria. However, by examining and comparing these two types of coffee extracts, the obtained results showed the Arabica coffee extract had more antimicrobial properties.

#### CONCLUSION

The results from the present study suggest that coffee ground extracts could be a potential source of bioactive compounds, thereby becoming a promising new functional food ingredient that can inhibit the growth of food-borne pathogen and food spoilage microorganisms.

**Keywords: Coffee Waste, Arabica, Robusta, Antimicrobial Properties**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### MDA-5 as a key sensor of SARS-CoV-2 infection: evidence from high expression and comparison with RIG-1

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The emergence of a novel coronavirus in Wuhan in 2019 precipitated a rapid spread to numerous nations, resulting in countless fatalities. It is indispensable to survey ratio and measure amount of high-risk groups infectious specially children with signs and symptoms.

#### MATERIALS AND METHODS

In this cross-sectional study we measured the amount of SARS-CoV-2 positive cases that collected from 122 nasal and throat swab samples in children under 5 years old with COVID-19 symptoms with Real-time PCR in Besat hospital. Although whole blood examinations were done to find out the expression of following inflammatory cytokines.

#### RESULTS AND DISCUSSION

In these 122 samples, we detected 27 (22.1%) positive cases confirmed by Real-time PCR. Of these 27 samples 17 (62.9%) cases were male and 10 (37.1%) cases were female children under 5 years old with COVID-19 symptoms. After examine whole blood in positive samples we found out significant differences in some of cytokines compare to healthy people, however there was no significant differences in IL-4 and IL-10 in healthy and patients but there was a little increase in positive samples compare to healthy group.

#### CONCLUSION

These finds shows male children under 5 years old with COVID-19 symptoms are in higher risk compare to female children with same conditions. After examine the whole blood of positive group and compared the results with control group we found out some inflammatory cytokines increased significantly in patients infected with COVID-19, also IL-4 and IL-10 were increased in patients compared to control group but not significantly.

**Keywords:** COVID-19, SARS-Cov-2, Children, Respiratory Infection.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of different concentrations of thionin and basic fuchsin media for typing of *Brucella* spp. biovars

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Brucellosis, “undulant fever”, is an infectious disease caused by the bacterial genus *Brucella*. Health management and timely diagnosis of brucellosis are of great importance for any effective control strategy in humans and animals. This study was aimed at investigating the appropriate concentrations of thionin and fuchsin dyes to identify the biovars of this bacterium.

### MATERIALS AND METHODS

A concentration of 0.4% of thionin and fuchsin dyes in distilled water, as well as serum dextrose, tryptic soy agar and Brucell agar were employed as basic media for making colored media. Thionin and basic fuchsin solutions were boiled in a bain-marie for one hour. Then concentrations of 10, 20, and 40 micrograms/ml for thionin dye and 10 and 20 micrograms/ml for fuchsin dye were made based on OIE instructions. Furthermore, the concentration of 0.1% dyes in the 2.5, 5, and 10 µg/ml for thionin dye, and 5 and 10 µg/ml for fuchsin were used for comparison. Horse serum was also considered as a factor stimulating the growth of *Brucella* bacteria in environments.

### RESULTS AND DISCUSSION

The findings presented in our study have demonstrated that a selective culture medium of *Brucella* agar along with serum is the best option for making colored media in the differentiation of *Brucella* biovars in classical typing. The percentage of dyes applied in colored media at the rate of 10, 20, and 40 µg/ml for thionin dye and 10 and 20 µg/ml fuchsin dye showed the best accuracy in differentiating different *Brucella* biovars. By increasing sterile and inactivated horse serum by 5%, bacterial growth increased compared to serum-free environments.

### CONCLUSION

Although serum dextrose, tryptic soy agar media have been used as basic media for making colored media in the past, the selective culture media of *Brucella* agar with horse serum, showed a high sensitivity for the growth of *Brucella* biovars, leading to better growth of *Brucella*.

**Keywords:** Thionin, Fuchsin, Typing, Biovar, *Brucella*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Effect of myxobacterial metabolite on differentiation of dental pulp-derived stem cell to odontoblasts

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Myxobacteria have gained recognition as one of the most prolific producers of bioactive secondary metabolites cover a wide range of chemical structures. In regenerative endodontics, odontogenic inducers are an important component of regenerative endodontics and play a significant role in promoting pulp cell differentiation and mineralization. This study aimed to evaluate the effect of myxobacterial metabolites on the differentiation of dental pulp stem cells into odontoblasts.

### MATERIALS AND METHODS

To obtain metabolites, the myxobacterial strain was inoculated to a 1/2 H fermentation medium, and its secondary metabolites were extracted using liquid-liquid extraction. The effect of metabolite exposure on pulp stem cell viability was assessed using the MTT assay. Odontoblastic markers expression was evaluated, and mineralized nodule formation was assessed using alizarin red staining, followed by quantitative measurement of calcium deposition. Additionally, the inhibitory effect of the metabolite extract on oral microbiota the standard broth dilution method (CLSI M07-A8) was evaluated.

### RESULTS AND DISCUSSION

The use of odonto/osteogenic inducers, such as growth factors, is an attractive strategy for promoting ossification and dental tissue repair. However, several challenges exist for the widespread use of growth factors in clinical settings, including their high physiological doses, short half-life, and severe side effects, such as aberrant bone formation, osteolysis, seroma, and retrograde ejaculation. The data showed that myxobacterial extract induces differentiation of dental pulp stem cells by enhancing the expression of Dentin sialophosphoprotein (DSPP) and Dentin matrix protein-1 (DMP-1), along with increasing calcium deposition. The results indicated that the nontoxic doses of 0.096 µg/ml and 0.048 µg/ml applied in odontogenic differentiation of stem cells did not inhibit the growth of oral microbiota. This research may lead to the discovery of new sources of medicinal compounds with the potential modality in regenerative dentistry and pulp capping materials for the maintenance of pulp vitality.

### CONCLUSION

The data suggest the potential therapeutic effects of myxobacterial secondary metabolites in regenerative endodontics and vital pulp therapy. Considering the importance of the oral microbiome to health, using dentinogenesis inducer compounds that have minimal impact on the normal resident oral microbiome is beneficial. In our follow up studies, the myxobacterial effective compounds having the signaling effect on differentiation will be isolated and characterized for both cytotoxicity and inducing effect.

**Keywords:** Odontogenic differentiation, Pulp mesenchymal stem cells, Myxobacteria, Secondary metabolites

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Expression and purification of recombinant chimeric PvpA-pMGA1.2 protein from *Mycoplasma gallisepticum* in *Escherichia coli*.

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

*Mycoplasma gallisepticum* is the primary agent of chronic respiratory disease in chickens, and infectious sinusitis in turkeys creating important economic losses in poultry industry, which is spread worldwide. pMGA and pvpA are two gene families of *Mycoplasma gallisepticum* that encode major surface proteins containing pathogenic, antigenic and immune evasion attributes. The objective of the present study was to express and purify the chimeric PvpA-pMGA1.2 recombinant protein from *Mycoplasma gallisepticum* for designing ELISA test.

#### MATERIALS AND METHODS

Dominant pattern with maximum covering was selected. The codon optimized sequence was cloned into the expression vector pET32a+ and transformed into *Escherichia coli* strain BL21(DE3), and the expressed protein was identified by SDS-PAGE.

#### RESULTS AND DISCUSSION

The results revealed that the recombinant chimeric PvpA-pMGA1.2 protein was highly expressed post- induction with 0.1 mM IPTG after 16 hours incubation at 37°C and protein was purified amount 138 mg per liter by affinity Batch formation method. The purified recombinant protein was confirmed by Western blotting.

#### CONCLUSION

This recombinant chimeric protein could be potentially used for the development of serodiagnostic test.

**Keywords:** rPvpA-pMGA1.2, *Mycoplasma gallisepticum*, Fusion protein

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Cloning and expression of a non-toxic mutant of *Clostridium perfringens* epsilon toxin, ETX-I51C

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Epsilon toxin (ETX), produce by *Clostridium perfringens* toxinotypes B and D, is the main cause of enterotoxemia. Enterotoxemia is one of the most important prevalent diseases in ruminants, specially sheep and goats. The non-toxic epsilon mutants are applicable for different purposes, especially enterotoxemia vaccine design. The aim of this study was to design and produce a non-toxic epsilon mutant, ETX-I51C, and to investigate its properties.

### MATERIALS AND METHODS

The I51C mutant of *C. perfringens* epsilon toxin was synthesized by site-directed mutagenesis (SDM) in the particular region of ETX gene with complementary mutagenic primers, based on the overlap-extension PCR. The ETX-I51C mutant was cloned in pET-26b(+) vector and the obtained recombinant pET-ETX-I51C plasmid was transformed into *Escherichia coli* (DE3). Positive clones were identified by plasmid extraction, cloning PCR, and sequencing. Protein expression of this mutant was induced by 0.5mM of IPTG and evaluated by SDS-PAGE, blotting, and ELISA.

### RESULTS AND DISCUSSION

Based on the sequencing results, the mutation was correctly created in the desired I51 position. The results showed that ETX-I51C mutant was successfully synthesized and cloned and its derivative toxoid could be well expressed in *E. coli*. According to ELISA results, the expression of ETX-I51C mutant was better than the control group and it had a better biological activity in the reaction with specific anti-ETX antibody.

### CONCLUSION

Therefore, the ETX-I51C mutant of *C. perfringens* epsilon toxin are suggested as suitable candidate for the later in vitro and in vivo immunologic investigations. Taken together, the results suggest that ETX-I51C is a potential vaccine candidate against enterotoxemia.

**Keywords:** *Clostridium perfringens*; Enterotoxemia; Epsilon toxin; Site-directed mutagenesis (SDM); ETX-I51C; Mutant

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Screening for the presence of the metallothionein gene in bacteria isolated from mines and industrial wastewater in Isfahan province, and investigating the biochemical and microbial characteristics of the isolated variants

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Excessive accumulation and circulation of heavy and toxic metals in the environment have raised environmental and health concerns. Nowadays, bioremediation processes utilizing natural microorganisms for detoxification of hazardous metals that pose threats to human health and the environment have gained attention. This study focuses on the detection of the metallothionein gene in bacteria isolated from mines in Isfahan. Evaluating the biochemical and microbiological characteristics of these bacteria can contribute to the identification of microbial strains with high bioremediation capabilities.

#### MATERIALS AND METHODS

Sampling and cultivation were conducted from approximately 90 different locations of iron smelting factories and stone mines in Isfahan. The colony PCR method was used for the detection of the metallothionein gene. The resistance level of the isolates to heavy metals was examined using MIC and MBC tests. All microbial characteristics, particularly drug resistance and biochemical properties, were evaluated. Ultimately, three highly resistant strains to cadmium, copper, and nickel were identified based on the 16S rRNA gene sequence and registered in the genomic database.

#### RESULTS AND DISCUSSION

The highest resistance levels of the strains to cadmium, copper, and nickel were calculated as 3.8, 2.6, and 1.5 milligrams per milliliter, respectively. Moreover, the resistance pattern of the metal-resistant strains to antibiotics indicated that most of them were also resistant to common antibiotics. Finally, one Gram-negative bacillus strain belonging to the *Pseudomonas* genus and two Gram-positive bacillus strains belonging to the *Bacillus* genus, all exhibiting high resistance to antibiotics and heavy metals, were identified and registered. Additionally, some biochemical characteristics showed significant differences between the metal-resistant bacteria and other variants.

#### CONCLUSION

Since microorganisms play a crucial role in bioremediation, tracking metallothionein-encoding genes in bacteria is of significant importance. High resistance to antibiotics in bacteria exhibiting higher resistance to heavy metals should be given attention.

**Keywords:** Metallothionein, Mines, Industrial effluents, Antibiotic resistance, Heavy metal

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Stability Evaluation of Ring Antigen Produced by RVSRI

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The Milk Ring Test (MRT) is valuable and useful in detecting *Brucella* antibody in milk. The evaluation of the stability of this antigen is used to confirm its effectiveness and its expiration date. It plays an important role in ensuring the quality of the product. Therefore, it is necessary to monitor the antigen ring test produced by Razi Vaccine and Serum Research Institute (RVSRI) and in order to determine the maximum shelf life of the antigen ring test

#### MATERIALS AND METHODS

100 sample vials of three series of antigen produced by RVSRI were performed in accelerated and long-term stability methods. In the accelerated stability studies, the products were examined at a temperature of 37 degrees for a period of up to 3 months, which is approximately equivalent to three years. Another method is to examine long-term stability studies, where the vaccine is placed in the recommended storage conditions of +4 to 36 months and +25 to 18 months. The following tests were performed including inactivity of *Brucella abortus* bacteria, the amount of mass per unit volume, the amount of phenol, pH level, purity and identity, the positive and negative control of the antigen.

#### RESULTS AND DISCUSSION

The results of the long-term stability studies showed that the antigen was confirmed and in accordance with the OIE standard. All the samples had similar results to the samples tested in the long-term evaluation method. This study showed that the MRT antigen produced by RVSRI was within the standard range in the time frame of the performed tests (2 and a half years), which lasts up to three years of study.

#### CONCLUSION

Based on years of experience, all the antigens produced by Razi Vaccine and Serum Research Institute can be used for years provided that they are kept in proper storage conditions (2 to 8°C) and that autoagglutination in the antigen has not been occurred.

**Keywords:** Antigen, Milk Ring Test, Stability, Brucellosis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Cloning and expression of the *Brucella melitensis* OMP28 and OMP31 genes in *Lactococcus Lactis* and investigations of immunogenic responses in BALB/c Mice

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Brucellosis is a common disease between human and animals in the world and the disease imposes a huge cost on the economy and society. There is still no suitable vaccine against this disease. Currently, the Live Brucella vaccines have disadvantages such as the inability to differentiate infected animals from vaccinated with classical Brucella tests in the first year, antibiotic resistance, the possibility of shedding the vaccine strain from milk and other animal secretions and the possibility of abortion of vaccinated animals. Finally, the virulence potential of common vaccine strains for humans led to the evaluation of the OMP31 and OMP28 recombinant proteins and their immunogenicity in the prevention of the disease.

### MATERIALS AND METHODS

In this study, OMP28 and OMP31 recombinant proteins were isolated from *Brucella melitensis* and cloned in *lactococcus lactis* NZ3900 vector. Colonies that have received the vector will turn yellow on this medium. After transformation of the genes to the host and the expression of the recombinant protein, mice were given the bacteria as an oral vaccines. Evaluation of the immunogenicity of the vaccine was performed orally in mice (2-5 x 10<sup>9</sup> cells for each mouse). Serum samples were collected from all mice on days 0, 7, 14, 21, 28 and antibody levels were measured against OMP28 by ELISA method.

### RESULTS AND DISCUSSION

The results showed a significant increase in the antibody titer of the vaccinated groups against OMP28 and OMP31 compared to the control group. It should be noted that all rats were weighed before the test and quarantined for two weeks for environmental compatibility in the laboratory.

### CONCLUSION

All these attempts to express this protein and investigate its antigenicity, indicating the high antigenicity of protein derived from OMP31 and OMP28 gene expression.

**Keywords:** Expression, OMP, Cloning, *Brucella Melitensis*, Immunogenicity.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### *Citrobacter braakii*, a new plant pathogen, causal agent of walnut decline

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Walnut canker is characterized by brown to blackish roundish blotches on the trunks and main branches, necrosis of inner bark and bleeding with dark brown to black-colored exudates. The present study aimed to identify the causative agents of walnut decline by their phenotypic features, approval of pathogenicity, the partial sequencing of the housekeeping genes in Razavi Khorasan.

#### MATERIAL AND METHODS

Ten Symptomatic samples were collected from walnut orchards of Razavi Khorasan in 2019. Pathogenicity of all isolated strains was carried out on walnut immature fruits cv. 'Hartley' and young green twigs of cv. 'Chandler'. All pathogenic strains were subjected to physiological, morphological and biochemical tests. 16S rRNA and housekeeping genes (*fusA*, *leuS*, and *pyrG*) were partially amplified and sequenced.

#### RESULTS AND DISCUSSION

Eight strains were able to cause necrosis and a dark-colored region in the mesocarp of immature walnut fruits and three representative strains caused necrosis on young inoculated twigs. Strains utilized starch, however, did not utilize esculin, Tween 20, Tween 80, and gelatin. The partial 16S rRNA gene sequence of strain KH7 indicated 99.63 % similarity to that of *Citrobacter braakii* ATCC5113<sup>T</sup>. The phylogenetic analyses based on the partial sequencing of three housekeeping genes, *fusA* (633 bp), *pyrG* (305), and *leuS* (640 bp) demonstrated that strains KH1, KH3, and KH7 belong to *C. braakii* species in a monophyletic clade with high bootstrap support.

#### CONCLUSION

To the best of our knowledge, this is the first report of *C. braakii* as a new plant pathogen which cause walnut decline. Identification of bacteria associated with walnut decline will eventually improve our understanding of the etiology of the disease and may result in improved management techniques for control.

**Keywords:** Emerging pathogens, Iran, *Juglans regia*, MLSA.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluation of relationship between Serum vitamin D level and acute febrile respiratory infections in children

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

About 4.5 million children die annually in the world due to acute respiratory diseases. Because of the importance of mortality in children, detecting the preventing factors of respiratory infections is very important. This study aimed to evaluation of relationship between Serum vitamin D level and acute febrile respiratory infections in children.

#### MATERIALS AND METHODS

In this case-control study, 50 children younger than 5 years old were studied in 2019. They were divided in two groups, acute febrile respiratory infection hospitalized in Allameh Behlool Gonabadi hospital (Gonabad- Iran) as case group and healthy children as control group. Data was entered into the checklist and analyzed by the software of SPSS version 16.

#### RESULTS AND DISCUSSION

In this study, there was a significant difference between the serum levels of vitamin D in case group and control group ( $P = 0.053$ ). The difference between vitamin D in sex ( $P = 0.25$ ), location ( $P = 0.48$ ), and attending in care center ( $P = 0.62$ ) were not significant. Vitamin D status in the consuming milk powder group ( $P = 0.005$ ) and the group with a history of repeated hospitalization ( $P = 0.004$ ), it was significantly higher.

#### CONCLUSION

The serum level of vitamin D in children with acute febrile respiratory infection hospitalized in hospital was higher than healthy outpatient children and this difference was significant.

**Keywords:** Respiratory tract infection, Children, Vitamin D

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Rational engineering to produce more thermostable carbonic anhydrase from *Sulfurihydrogenibium yellowstonense* for industrial application

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Carbon dioxide (CO<sub>2</sub>) capture and storage (CCS) processes play a crucial role in mitigating climate change by reducing CO<sub>2</sub> emissions. Thermostable carbonic anhydrases (CAs) have shown great potential in enhancing the rate of CO<sub>2</sub> absorption into solvents, offering a promising avenue for robust and cost-effective industrial applications. This study focuses on rational engineering of a thermostable CA (SspCA) derived from the thermophilic bacterium *Sulfurihydrogenibium yellowstonense*, aiming to improve its thermostability while maintaining catalytic efficiency under high-temperature conditions.

### MATERIALS AND METHODS

In this investigation, in silico tools were employed to rationally design a mutant form of the SspCA enzyme. The joint candidate from the three tools, RosettaDesign, PoPMuSiC and FoldX, with the best score was selected for construction using the site-directed mutagenesis method. The mutant, was analyzed to assess its impact on the thermostability and catalytic activity of the CA. Molecular dynamics (MD) simulations were utilized to gain insight into the structural changes caused by the mutation and understand its effects on local flexibility and stability.

### RESULTS AND DISCUSSION

The rational design of the K100G mutation resulted in improved thermostability of the CA. Although the general folding and catalytic efficiency of the enzyme were preserved, the melting temperature of the K100G mutant increased by 3°C. Notably, the half-life of CO<sub>2</sub> hydration activity at 85°C was extended twofold, making it more suitable for industrial processes carried out under elevated temperatures. Molecular dynamics simulations revealed that the K100G mutation induced a reduction in local flexibility of the protein. This effect was primarily achieved by the rearrangement of salt bridges and hydrogen interaction networks, leading to structural rigidification in the neighboring regions.

### CONCLUSION

This study demonstrates the potential of rational engineering in enhancing the thermostability of enzymes like carbonic anhydrases, essential for efficient CO<sub>2</sub> capture and storage processes in high-temperature industrial settings. The K100G mutation in the SspCA enzyme indirectly resulted in increased thermostability by confining the flexible parts and promoting local structural rigidity. These findings highlight the importance of targeted mutations to optimize the performance of biocatalysts for industrial applications, ultimately contributing to the advancement of carbon capture technologies and sustainable environmental practices.

**Keywords:** Thermostability, Rational protein design, Carbonic anhydrase, CO<sub>2</sub> sequestration, MD simulation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### *Enterobacter hormaechei* subsp. *hoffmannii*, a new plant pathogen, associated with walnut decline

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Persian walnut (*Juglans regia*) has a considerable economic importance worldwide. However, the vigor and vitality of walnut trees were heavily affected by bark canker during the last few years. Stem tissue necrosis, and bleeding with black-colored exudates walnut trees were observed in Markazi province in 2018.

#### MATERIAL AND METHODS

A total of eight symptomatic samples were collected from affected walnut trees. The pathogenicity of all strains was proved by inoculating a suspension of the bacterial strains under the bark of immature walnut fruits cv. 'Hartley'. Conventional phenotypic assays were performed. 16S rRNA, *gyrB*, and *infB* genes were partially amplified and sequenced.

#### RESULTS AND DISCUSSION

Sixteen strains with a metallic green sheen were isolated on EMB-agar medium. Eleven strains caused necrosis and a dark-colored region in the mesocarp around the inoculation site 14 days post-inoculation. Moreover, three representative strains induced necrotic and black-colored tissues in the bark of young green twigs of two-year old walnut seedling cv. 'Chandler'. No symptoms were detected in the fruits and young branches infiltrated with sterile distilled water. Strains hydrolyzed gelatin, however, did not utilize starch, esculin, Tween 20 and Tween 80. These strains produced indole and urease. The partial 16S rRNA gene sequence of strain MR1 was identical to that of *Enterobacter hormaechei* subsp. *hoffmannii* DSM 14563<sup>T</sup>. The maximum-likelihood phylogenetic tree was created using concatenated sequences of *gyrB* (684 bp) and *infB* (906 bp) genes. The strains MR1, MR3, and MR5 were grouped with *E. hormaechei* subsp. *hoffmannii* DSM 14563<sup>T</sup>.

#### CONCLUSION

To the best of our knowledge, this is the first report of *E. hormaechei* as plant pathogen. This study highlights the fact that walnut decline has to be considered a complex disease in which several bacteria are involved.

**Keywords:** Emerging pathogens, Iran, *Juglans regia*, MLSA.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The role of APH (3')-VIa and ANT (2'')-Ia enzymes in increasing the minimum inhibitory concentration of aminoglycosides against clinical isolates of *Acinetobacter baumannii*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Acinetobacter baumannii*, is an opportunistic pathogen known for causing a variety of infections in humans. Aminoglycosides are the significant antibiotics to treatment the infections caused by *Acinetobacter baumannii*. This study aimed to determine the role of genes encoding aminoglycoside-modifying enzymes (AMEs) in increasing the minimum inhibitory concentration of aminoglycosides against clinical isolates of *A. baumannii*.

### MATERIALS AND METHODS

This study was performed on *A. baumannii* isolated from the patients. We collected 100 clinical isolates of *A. baumannii* and identified by microbiological standard tests. The presence of APH (3')-VIa (*aphA6*) and ANT (2'')-Ia (*aadB*), AME encoding genes, was detected by PCR method.

### RESULTS AND DISCUSSION

The most effective aminoglycosides in this study were netilmicin with 68% resistance rate, while 94% of the isolates were resistant against gentamicin, kanamycin, tobramycin, and streptomycin. Also, 77% and 73% of the *A. baumannii* clinical isolates in this study were contained the *aphA6* and *aadB* genes. However, 39 *A. baumannii* isolates contained simultaneously both *aphA6* and *aadB* genes. Among them, (97.4%), (97.4%), (87.1%), (94.8%), (100%), (84.6%), and (82%) isolates were non-susceptible to gentamicin, tobramycin, amikacin, netilmicin, kanamycin, streptomycin, and spectinomycin, respectively. In addition, among 77 isolates containing the *aphA6* gene, (87.01%), (92.2%), (74.02), (64.93%), (88.31%), (93.5), and (76.62%) isolates showed a MIC range of  $\geq 256$   $\mu\text{g/ml}$ , respectively. However, among 73 isolates carrying the *aadB* gene, (95.82%), (93.15%), (65.75%), (65.75%), (87.67%), (94.52%), and (86.3%) isolates exhibited this MIC range.

The analysis of AMEs encoding genes in this study revealed a significant occurrence of *aphA6* and *aadB* genes, aligning with previous research conducted in Iran.

### CONCLUSION

High-level aminoglycoside MIC ranges in isolates with the simultaneous presence of AME encoding genes indicated the importance of these genes in resistance to aminoglycosides.

**Keywords:** *Acinetobacter baumannii*, Aminoglycoside Modifying Enzymes, Aminoglycoside resistance.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Bioinformatics and clinical study of miR-200 family members on E3 ubiquitin genes and KRAS in PI3K/AKT and MAPK/WNT signaling pathways in breast adenocarcinoma

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

Breast cancer is one of the most common cancers in women in both developed and developing countries, and it is the main cause of cancer-related death among women. And the main cause of death is cancer among women. However, if it is detected in the early stages, it can be treated. Due to the greater understanding of the molecular pathways involved in the pathogenesis of cancers, several macromolecules at the level of RNA, DNA and protein have been investigated as biomarkers in the diagnosis and prognosis of cancers. Therefore, the members of the miR-200 family are one of the oncogenic miRNAs in breast cancer that are associated with increased expression, which we will discuss with the clinical and bioinformatics studies of the members of the miR-200 family on the target genes of E3 ubiquitinase and KRAS in intracellular signaling pathways.

### MATERIALS AND METHODS

Accordingly, the paraffin blocks used for this study include 30 breast cancer paraffin blocks and 30 normal paraffin blocks. The average age of the samples was between 45 and 60 years, and the progress and stage of the disease is in Stage II and Stage III, and then extracting RNA from healthy and normal tissue samples, and then extracting the corresponding miRNA with a special kit and detecting quantitative RNA and cDNA synthesis, and finally measuring the expression level. The target genes and miRNAs were analyzed by Real time PCR technique.

### RESULTS AND DISCUSSION

In this study, the miR-200 family caused the average KRAS gene expression to increase by 1.4 times ( $P < 0.05$ ) in patients with breast cancer compared to healthy individuals by examining the  $\Delta\Delta C_t$  method, but the level of E3 gene expression in breast cancer patients. Compared to healthy people, it has decreased by 3.4 times ( $P < 0.05$ ).

### CONCLUSION

Finally, the results obtained from this study Clinical/bioinformatics showed that among the members of the MIR-200 family (MIR-200b and MIR-141) with the aim of placing E3 and KRAS genes in important biological processes and molecular pathways involved in Breast cancer with expression profile changes in tumor tissue samples can be used as a biomarker. to be diagnostic/prognostic in breast cancer.

**Keywords:** Mir-200, E3ubiquitin Genes, KRAS, Breast Adenocarcinoma, Real Time PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Distribution of Plasmid-Mediated Quinolone Resistance (PMQR) in Uropathogenic *Escherichia coli* isolated from UTIs in North of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Escherichia coli* is the leading etiological agent of community-acquired urinary tract infection (UTI). Quinolones have long been the choice of empirical treatment for UTIs. Plasmid-mediated quinolone resistance (PMQR) is important not only for conferring resistance to quinolones but also because of the presence of *PMQR* genes on plasmids carrying genes encoding resistance to other antimicrobials. In this study, we aimed at investigating the frequency of *PMQR* genes in quinolone-resistant and/or extended-spectrum beta-lactamase (ESBL)-producing *E. coli* strains isolated from adult patients diagnosed with UTI.

### MATERIALS AND METHODS

In this cross-sectional study, 77 UPEC strain were isolated from UTI patients who were admitted to hospitals affiliated with Babol University of Medical Sciences, north of Iran, during the period of 2021-2022. The confirmation of isolates was done through standard microbiological and biochemical tests. The presence of *PMQR* genes (*qnrA*, *qnrB*, *qnrS*) was investigated by conventional PCR analysis.

### RESULTS AND DISCUSSION

The results of PCR amplification of the *PMQR* genes revealed that 42.9% (67/77) of the isolates harboring at least one *PMQR* genes with the following distributions: 44.2% (34/77) *qnrS* gene and *qnrB* 11.7% (9/77). While, none of the isolates harbored the *qnrA* gene. The results are presented in Table 1.

Variable	Adults/ N= 77/ No (%)	P-value
<i>qnrA</i>	-	-
<i>qnrB</i>	9 (11.7)	0.56
<i>qnrS</i>	34 (44.2)	0.07

### CONCLUSION

PMQR determinants were found to be widespread among our isolates. In this regard, the *qnrS* gene was the most frequent gene. Therefore, a regular periodic monitoring program and rational antimicrobial use is needed to control and prevention of antibiotic resistance phenomenon and contributed genes among UTI-causing *E. coli* isolate in adult populations.

**Keywords:** *Escherichia coli*, UPEC, quinolone resistance, plasmid

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Phylogenetic Group Distribution of Uropathogenic *Escherichia coli* isolated from UTIs in North of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The study of the prevalence of the phylogenetic group in the uropathogenic *Escherichia coli* (UPEC) strain isolated from patients with Urinary tract infections (UTIs) is valuable for epidemiology aspects. UPEC is divided into different phylogenetic groups that differ in their antibiotic resistance patterns, serogroups and pathogenicity. therefore, the current cross-sectional study aimed to investigate the prevalence of the phylogenetic group in UPEC isolates obtained from patients with UTIs in north of Iran.

### MATERIALS AND METHODS

In this cross-sectional study, 77 UPEC strain were isolated from UTI patients who were admitted to hospitals affiliated with Babol University of Medical Sciences, north of Iran, during the period of 2021-2022. The confirmation of isolates was done through standard microbiological and biochemical tests. Phylogenetic groups were determined by the quadruplex PCR method.

### RESULTS AND DISCUSSION

Based on the quadruplex PCR assay findings, a total of 71 of 77 UPEC isolates were assigned to 6 of the 7 phylogenetic groups and the results are presented in Table. Accordingly, the majority of UPEC isolates belonged to phylogenetic group B<sub>2</sub>, accounting for 42.9% of the total sample, followed by groups E (16.9%), group A and D (9.1%), F (7.8%) and B<sub>1</sub> (6.5%). Notably, none of the isolates tested positive for phylogenetic group C. Of the remaining isolates, 7.8% were found to be untypeable.

Variables	Adults/N= 77/ No (%)	P-value
A	7 (9.1)	0.12
B <sub>1</sub>	5 (6.5)	
B <sub>2</sub>	33 (42.9)	
D	7 (9.1)	
E	13 (16.9)	
F	6 (7.8)	
Unknown	6 (7.8)	

### CONCLUSION

Our findings indicated that phylogroup B<sub>2</sub> and group E were the most predominant phylogenetic groups among UPEC isolates in our region of Iran which is comparable with other parts of the world.

**Keywords:** *Escherichia coli*, UPEC, phylogenetic group, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Mushrooms native to Iran: Opportunities and challenges for therapeutic and pharmaceutical research

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Wild mushrooms have long been appreciated for their nutritional and medicinal properties. Although the number of mushrooms species grown commercially are very limited, there are at least 14,000 taxonomically known wild mushroom species worldwide. Various studies have also reported the occurrence of wild macrofungal taxa in Iran, especially in temperate forests. This review addresses the opportunities and challenges of working with Iranian wild mushrooms.

### MATERIALS AND METHODS

The basic data presented here are the consolidated published findings of about 10 years of experimental research on Iranian wild mushrooms at ACECR-Khorasan Razavi Branch.

### RESULTS AND DISCUSSION

Accurate authentication of collected wild mushrooms, preserving pure mycelia, and if applicable, adopting into locally available lignocellulosic substrates (to produce fruiting bodies) are essential to ensure access to fungal materials and ensure reliability and reproducibility for further biomedical, biochemical, and omics studies. Domesticated wild mushrooms may also promote further commercial-scale production of edible fruiting bodies and their use in the food industry. Based on the aforementioned strategy, our efforts have led to collection of over 100 Iranian wild mushroom species, which have been well-authenticated and deposited on Genbank. Apart from applicable findings on growth characteristics of these wild mushrooms in different lignocellulosic substrates, we have shown their antimicrobial, anticancer, antioxidant and immunomodulatory activities, and nanoparticles biosynthesis capabilities both in vitro and in vivo. Extraction, purification, structural characterization, and proximate analysis approaches have also been established. In particular, we have investigated the differences in the linear and spatial structure of beta-glucans between wild and commercial Enoki mushrooms. However, there are still limitations and challenges. Apparently, many Iranian wild mushrooms in our biobank remain unexplored. It also appears that successive cultivation of domesticated wild mushrooms in artificial substrates (rather than in their natural habitat) can lose their ability to produce secondary metabolites required to defense against pathogens. Extensive research has been done on metabolomics and, to a lesser extent, proteomics. Nevertheless, information on changes at genes and mRNA levels is very limited.

### CONCLUSION

Iranian wild mushrooms can be considered a valuable source of novel bioactive lead compounds. However, multidisciplinary and in-depth studies are required to reveal their potency in the healthcare and the food industry.

**Keywords:** Iranian wild mushrooms, authentication, domestication, nutritional and medicinal properties, omics,

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Identification of probiotic bacteria isolated human colostrum milk and invitro investigation their antimicrobial effect on the infectious agents of children diarrhea

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Breast milk is an important factor in the initiation and composition of the neonatal flora gut microbiota which contains potentially probiotic lactic acid bacteria. Lactic acid bacteria are very significant to human health due to the production of some antimicrobial substances and ability to inhibit pathogenic bacteria. There is an increasing trend in the field of research into the characterization of new acid lactic isolates with potential application to the health and disease prevention. Accordingly, the present study isolated probiotic bacteria from human colostrum milk to analyze their antimicrobial potential on agents of children diarrhea.

### MATERIALS AND METHODS

In this study, isolates were assayed to phenotypic characteristics, in vitro viability different temperatures and high salt concentration, resistance to gastrointestinal simulated conditions, the antimicrobial activity of antibiotics and as well, their antimicrobial activity were surveyed on 4 of diarrheagenic bacteria, *Escherichia coli* 0157:H7 (ATCC 43890), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhi* (PTCC1609) and *Shigella dysentery* (ATCC 13313).

### RESULTS AND DISCUSSION

Based on the results, 4 of isolates from 6 lactobacilli isolates and 7 isolates from 34 Enterococci isolates had capacities survive under the mentioned conditions. Through molecular identification, 2 Lactobacillus isolates belonged to *L. fermentum* species and the other 2 isolates belonged to *L. plantarum* and *L. salivarius* species. The most inhibitory effect was observed in C1 isolate from enterococci and Q2b (*L. fermentum*) isolate from lactobacilli on enterohemorrhagic *E. coli* and *S. aureus*, respectively.

### CONCLUSION

The identified lactic acid bacteria as probiotics could be used in formula infant to protect children health versus infectious diseases such as diarrhea after the legal procedures and to get necessary safety.

**Keywords:** Antimicrobial effect, Colostrum, Diarrhea, Lactic acid bacteria, Probiotic

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Detection of antibiotic resistance profile of *E. coli* strains producing Extended-spectrum beta-lactamases isolated from patients referred to Allame-Bohlol Gonabadi hospital

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Antibiotic resistance in pathogens, especially *Escherichia coli*, has become a major treatment issue. Resistance of this organism occurs via the production of extended-spectrum  $\beta$ -lactamases (ESBLs). The aim of this study was to determine the prevalence of the *E. coli* strains producing extended-spectrum beta-lactamases (ESBLs) enzymes and their antibiotic resistance patterns.

### MATERIALS AND METHODS

In this descriptive study, 255 different *Escherichia coli* isolates isolated from the samples of patients referred to Allameh Bohloul Hospital in Gonabad were examined. After collection, the data were entered in SPSS software version 16 and analyzed using descriptive statistics tests.

### RESULTS AND DISCUSSION

In this study, out of 255 people sampled, 83.5% were female and the mean age of patients was  $39 \pm 30$  year. The frequency of *Escherichia coli* producing ESBL (Extended spectrum beta-lactamase enzymes) was 40.78%. The highest susceptibility to imipenem (81.70%) and the highest resistance to cefotaxime (100%) were observed. The frequency of isolates with ESBL in outpatients was 34.01% and inpatients was 50% ( $P = 0.01$ ). The number of isolates with ESBL was higher in men than women ( $P = 0.018$ ). 68.26% of ESBL generating isolates had multidrug resistance and 64.42% had cross-resistance.

### CONCLUSION

The results showed that more than 40 % isolates were detected as ESBL- producing strains. Based on the observed high percentage of resistance to the third-generation-cephalosporins, it is vitally important to perform precise and accurate antibiogram. Avoiding excessive over-prescription of antibiotics for infections caused by ESBL-producing organism is an inevitable necessity.

**Keywords:** Antibiotic resistance, *Escherichia coli*, Extended-spectrum beta-lactamase enzyme

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Cultivation of *Chlorella* sp. S4 in an open raceway pond to increase biomass and carbohydrate production scale for bioethanol production

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Microalgae are photosynthetic microorganisms with a high growth rate, high carbon fixing capability, and a short harvesting cycle. Some species have been identified as suitable sources for producing bioethanol due to their high carbohydrate content. Nutritional and environmental factors, cultivation systems (closed or open systems), and strain selection can affect the biomass and carbohydrate production of microalgae. To release the carbohydrate content, a pre-treatment procedure is necessary. Through the biological fermentation of carbohydrates, bioethanol is produced. This study aims to cultivate a native Persian Gulf isolate in an open raceway pond to increase biomass and carbohydrate production scale for bioethanol production.

### MATERIALS AND METHODS

Microalga cells were cultivated in a 200 L indoor open raceway pond containing 150 L of modified Rudic's medium with a salinity of 35 g/L. The growth was done under a photoperiod of 16 h light / 8 h dark, light intensity of 9000 lux, at 25 °C for 16 days. Stirring was produced by a paddle wheel at the speed of 13 rpm. The dry biomass was pretreated using 3% (v/v) of H<sub>2</sub>SO<sub>4</sub> at 121 °C for 20 min. *Saccharomyces cerevisiae* (TT) cells were inoculated into a synthetic fermentation medium enriched with microalga hydrolysate and maintained at 30 °C for 48 h for ethanol production.

### RESULTS AND DISCUSSION

The microalga was able to reach its maximum dry biomass of 1 g/L, and carbohydrate concentration of 0.3 g/L in an open raceway pond. The highest ethanol concentration of 8.1 g/L (yield of about 0.41 g ethanol/g consumed carbohydrate) was observed after 48 h. As a result, bioethanol produced from microalgae could be used in the future as a source of green energy.

### CONCLUSION

Microalgae cultivation activities to produce bioenergy or other purposes could become a significant income for local people.

**Keywords:** Bioethanol, Carbohydrate, Microalgae, Open raceway pond

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### *Streptococcus agalactiae* antibiotic resistance in pregnant women in Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Streptococcus* (S.) *agalactiae* colonizes in the female genitourinary and lower gastrointestinal tracts and is responsible for a wide range of infections in newborns, pregnant women and non-pregnant adults. Therefore, antibiotic prophylaxis and infection treatment against S. *agalactiae* is important. The aim of this study was to determine the prevalence of S. *agalactiae* antibiotic resistance in Iranian patients, especially among pregnant women.

#### MATERIALS AND METHODS

A systematic literature search was conducted in PubMed, Scopus, Google Scholar and the Scientific Information Database (SID) databases by using related keywords and without any time limitation.

#### RESULTS AND DISCUSSION

A total of 26 studies reporting the prevalence of S. *agalactiae* antibiotic resistance in Iran met our predefined inclusion and exclusion criteria and were included in the meta-analysis. High rates of S. *agalactiae* antibiotic resistance in pregnant women were found against tetracycline (96.2%), trimethoprim-sulfamethoxazole (84.7%), cefotaxime (41.3%), clindamycin (26.8%) and erythromycin (21%). Additionally, resistance to penicillin (4.2%), ampicillin (2.7%), cefazolin (7.6%), vancomycin (2.4%), ceftriaxone (12.5%), ciprofloxacin (13.6%) and nitrofurantoin (0%) was low.

#### CONCLUSION

Our results revealed that penicillin and ampicillin among penicillin-tolerant Iranian pregnant women, and vancomycin and cefazolin among penicillin-allergic women are still drugs of choice in intrapartum prophylaxis for preventing S. *agalactiae* vertical transmission and early-onset neonatal disease.

**Keywords:** Antibiotic resistance, *Streptococcus agalactiae*, Iran.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Educational Needs Assessment of Medical and Midwifery Students about Prevention of Mother-to-Child Transmission of HIV

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

One of the most important ways of transmitting acquired immunodeficiency syndrome (AIDS) is vertical transmission or mother-to-child transmission (MTCT). The medical and midwifery students are also among the health care workers (HCWs) who interact very closely with the pregnant women during the parturition process. Therefore, this study was conducted to assess the educational needs of medical and midwifery students of Gonabad University of Medical Sciences on the prevention of mother-to-child AIDS transmission.

### MATERIALS AND METHODS

This cross-sectional study was conducted in 2019 on 120 medical (extern and intern) and midwifery (semester 4 and above of bachelor and master) students of Gonabad University of Medical Sciences. Participants completed three questionnaires of demographic variables, the perceived needs questionnaire on MTCT, and the real needs questionnaire on MTCT of AIDS. Data were statistically analyzed using SPSS version 23 and 0.05 was considered as a significant level.

### RESULTS AND DISCUSSION

The majority of participants were female (77.5%) and single (65%). In this study, 48.3% and 51.7% were medical and midwifery student, respectively. Sixty-three and a half percent of medical student and 36.5% midwifery student had high real educational need, respectively. More than half of the participants in this study (59.2%) felt a great need for education on mother-to-child AIDS transmission. Among the areas of real educational need, the highest scores were related to the area of prevention and the lowest score was related to the area of symptoms. The highest percentage of participants with high real need were training in higher semesters ( $p = 0.015$ ). The percentage of people who had a real need was higher among medical students than midwifery ( $p = 0.004$ ).

### CONCLUSION

According to the high real and perceived needs of students, especially in the higher semesters and the field of medicine, a re-examination of the educational curriculum is required.

**Keywords:** AIDS, HIV, Mother-to-child transmission, perceived educational needs, real educational needs, needs assessment

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### The *Chlamydia trachomatis* genotypes isolated from women with cervicitis in Kamali Hospital of Karaj (Iran)

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Chlamydia trachomatis* is considered to be one of the main pathogens of venereal diseases. There is no data concerning the frequency and genotyping of *C. trachomatis* in Iranian women who had cervicitis referred to Kamali Hospital of Karaj city and estimate the potential risk factors for this sexually transmitted disease.

#### MATERIALS AND METHODS

Cervical samples collected from 201 women with initial diagnosis of cervicitis (mean age 21.5 years) were analyzed through molecular assay for the detection of Chlamydial infection. Phylogenetic analysis on the *ompA* gene was performed on positive samples to identify the corresponding genotype.

#### RESULTS AND DISCUSSION

Out of 201 samples, 114 (56.7%) samples were *C. trachomatis* positive. Twentyfive positive samples were sequenced randomly. The genotype L2 (24 [96 %]), D (1 [4%]) were identified. Interestingly, none of the patients who were positive to genovar L2 had symptoms of lymphogranuloma venereum (LGV).

#### CONCLUSION

In comparison to similar researches, our results show a higher prevalence of *C. trachomatis* infection in female patients with cervicitis in Karaj. The findings also emphasize on diagnostic tests for *C. trachomatis* infection should be included in the screening programs for women.

**Keywords:** *Chlamydia trachomatis*, Cervicitis, Genotype, *omp1*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation of virulence factors and genes associated with biofilm and protease in *Stenotrophomonas maltophilia* isolates in Bushehr, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Stenotrophomonas maltophilia* is a nosocomial pathogen. The pathogenesis of *S. maltophilia* infections involves several virulence factors. The ability to form biofilm is one of the important characteristics of this organism. The aim of this study was to investigate the virulence factors and genes associated with biofilm and protease in *S. maltophilia* isolates in Bushehr, Iran.

### MATERIALS AND METHODS

Eighty-seven *S. maltophilia* isolates (67 clinical isolates and 20 environmental isolates) were investigated. The clinical isolates were collected from three hospitals and the environmental isolates were collected from two hospitals and one dental clinic. The isolates were examined for the production of virulence factors including DNase, hemolysin, protease, gelatinase, lipase, lecithinase, hyaluronidase, and biofilm. To detect *rmlA*, *rpjF*, *spgM*, *smf-1*, *StmPr1* 868 bp, *StmPr1* 1621 bp, and *StmPr2* genes, PCR and sequencing was carried out.

### RESULTS AND DISCUSSION

All isolates (100%) produced DNase, hemolysin, protease, lipase, and hyaluronidase. Seventy-eight (89.7%) isolates were gelatinase producers, and 85 (97.7%) isolates were lecithinase producers. All isolates were biofilm producers: 79 (90.8%) isolates produced strong biofilm, 5 (5.7%) isolates produced moderate biofilm, and 3 (3.5%) isolates produced weak biofilm. The frequency of *smf-1*, *rmlA*, *rpjF*, and *spgM* was 93.1%, 86.2%, 26.4%, and 59.8%, respectively. The frequency of protease genes including *StmPr1* 868 bp, *StmPr1* 1621 bp, and *StmPr2* genes was 12.6%, 41.4%, and 18.4%, respectively. The results of our study showed that the frequency of isolates producing DNase, hemolysin, protease, gelatinase, lipase, lecithinase, hyaluronidase, and biofilm is high in Bushehr. In this study, all the isolates that had *spgM* or *rpjF* or both genes were strong biofilm producers. It should be noted that the presence of isolates that lacked *spgM* and *rpjF* genes, but produced strong biofilm, indicates that in addition to these two genes, other genes or factors may play a role in the production of strong biofilm. *StmPr1* 1621 bp had a higher frequency among all 87 isolates and also among clinical isolates compared to *StmPr1* 868 bp and *StmPr2*.

### CONCLUSION

Based on the results of this study, *S. maltophilia* in our region is capable of producing several factors including enzymes which might play roles in pathogenicity.

**Keywords:** *Stenotrophomonas maltophilia*, biofilm, protease, *rpjF*, *spgM*, *rmlA*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of asymptomatic bacteriuria, antibiotic resistance pattern and related factors in pregnant women referring to Gonabad Community Health Centers

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Urinary tract infection is the most common bacterial infection during pregnancy that can present as asymptomatic bacteriuria, cystitis or pyelonephritis. This study was performed to evaluate the prevalence of asymptomatic bacteriuria, antibiotic resistance pattern and related factors in pregnant women referring to Gonabad community health centers in 2019.

### MATERIALS AND METHODS

This study is a cross-sectional study. In this study, 295 pregnant women referring to Gonabad community health centers participated from April 2019 to March 2020. Laboratory and demographic information of the samples were extracted from the Sib system and laboratories data base in Gonabad. Statistical analysis was performed using SPSS 16.0 software and descriptive and inferential statistics. The acceptable p-value was considered 0.05.

### RESULTS AND DISCUSSION

The results showed that 10.5 % of pregnant women had asymptomatic bacteriuria. Significant relationship was observed between asymptomatic bacteriuria and history of abortion ( $P=0.018$ ), anemia ( $P=0.008$ ) and age ( $P=0.012$ ), but no significant relationship was observed between asymptomatic bacteriuria and the manner of last delivery, gravidity, multi-fetal condition, body mass index, education, occupation and blood group ( $P> 0.5$ ). The most common organism of asymptomatic bacteriuria was *Escherichia coli* (67.7%) and then Coagulase-negative Staphylococcus (16.1%). The most appropriate antibiotic was Nitrofurantoin, to which 90.3% of organisms were sensitive.

### CONCLUSION

Asymptomatic bacteriuria in pregnancy is a common problem in Gonabad. Due to its side effects, it is recommended to re-culture urine in high-risk individuals at regular intervals during pregnancy and treat it with appropriate drugs to reduce maternal and fetal complications.

**Keywords:** Asymptomatic bacteriuria, Antibiotic resistance pattern, Pregnancy

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Prevalence of group B streptococcus colonization in the vagina of pregnant women referred to Fatemie hospital in Hamadan city and related factors

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Group B streptococcus (GBS) is one of the relatively common causes of vaginal infection in women of reproductive age. Considering the different prevalence of GBS in different geographical locations, this study was conducted with the aim of determining the prevalence of GBS colonization in the vagina of pregnant women referred to Fatemeh hospital in Hamadan city in 2021.

#### MATERIALS AND METHODS

In this descriptive-analytical study, 130 pregnant women referred to Fatemiyeh hospital in Hamadan city in 2021 were examined for GBS colonization using the purpose-based sampling method. Sampling was done using a sterile swab from the vaginal area, and the swabs were transferred to the microbiology laboratory after being placed in the Todd Hewitt broth medium. Bacterial diagnostic tests were performed to identify GBS. The results were analyzed with SPSS version 16 software.

#### RESULTS AND DISCUSSION

The average age of the studied women was  $29.85 \pm 6.86$  years. About 70% of them had a history of pregnancy at least once, 30% had a history of abortion, 6.2 % had a history of premature birth, 6.9% had a history of gestational hypertension, 16.4 % had a history of gestational diabetes, 3.1 % had a history of genital disease, and 47.7 % had a history of urinary tract infection. The prevalence of GBS was 3.8 %. No significant relationship was observed between the frequency of GBS with the age of pregnant women, gestational age, previous pregnancy records, history of abortion and premature birth, and their disease records.

#### CONCLUSION

Although the colonization of GBS in the vagina of pregnant women referred to Fatemiyeh hospital in Hamedan was low, due to its adverse effect on the outcome of pregnancy, screening for GBS infection and, as a result, its timely diagnosis and treatment is of special importance.

**Keywords: Group B Streptococcus, Colonization, Pregnant Women**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Application of microbial toxins to model neuroinflammatory diseases in animals

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Modeling diseases in laboratory animals is considered one of the important stages of research in the development of new therapeutic solutions. Bacterial lipopolysaccharide (LPS) as a bacterial typical endotoxin, is widely used to establish neuroinflammatory animal models associated with neurodegeneration (e.g., Alzheimer disease: "AD"). The completion of studies on neurodegenerative diseases requires the creation of animal and cellular models of these diseases. LPS could apply for a long time with different methods, doses and types for this purpose.

### MATERIALS AND METHODS

Route of LPS administration include intracerebroventricular (ICV), intraperitoneal (IP) or intravenously (IV). Some of these methods are performed with simple injections, while others are performed with surgery and the use of specialized devices such as stereotaxic setups.

### RESULTS AND DISCUSSION

It is important to understand mechanistic details of neuroinflammation formation because studies showed gut microbiota communicates with the central nervous system (CNS) via called gut-brain axis. As far LPS consider as one of major risk factors for sporadic AD development, investigation on gut microbiota and local/systemic presence of LPS to form neuroinflammation is become more important nowadays. A very important point in creating a model of neuroinflammation in a laboratory animal is that all types of commercial and extracted endotoxins can have different effects on the duration of its development and durability.

### CONCLUSION

Different route of LPS administration to induce neuroinflammation, dose dependent treatment of animals with LPS, Type and age of animal, the solvent used in injections, the purity of LPS and even the origin of LPS used can be effective in the type of neuroinflammation model created and its durability. Using LPS is found more affordable strategy and easier method rather than conventional methods of neuroinflammation modeling in animals like using beta-amyloid (A $\beta$ ).

**Keywords:** Bacterial endotoxin, Lipopolysaccharide,

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Molecular investigation of *Brucella* in Iranian women with spontaneous miscarriage

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Spontaneous miscarriage is a significant health concern in Iran, affecting many women. Several factors, including infections, can contribute to spontaneous miscarriages. *Brucella* is one of the bacterial agents that can have adverse effects on pregnancy outcomes. Therefore, this study aims to investigate the presence of *Brucella* in abortion samples obtained from women who experienced spontaneous miscarriages across various provinces in Iran.

#### MATERIALS AND METHODS

A total of 728 abortion samples, comprising placenta and cotyledon tissues, were collected between March 2021 and March 2022 from 409 women who experienced spontaneous miscarriages in Tehran, Fars, and West Azerbaijan provinces of Iran. DNA extraction was performed on the abortion samples, followed by a quantitative real-time PCR (qPCR) assay targeting specific fragments of the IS711 elements to molecularly identify *Brucella*. Furthermore, a qPCR assay utilizing specific probes and primers for *Brucella melitensis* and *Brucella abortus* was conducted to determine the particular species present.

#### RESULTS AND DISCUSSION

The overall prevalence of *Brucella* species in women with spontaneous abortion was 0.24% (1/409). A specific strain identified in this study was *Brucella melitensis*, belonging to a pregnant woman who had no previous history of miscarriages and resided in rural areas of West Azerbaijan province.

#### CONCLUSION

While the low prevalence rate of *Brucella* spp. in spontaneous miscarriages suggests that it may not be a significant contributor in most cases, the identification of *Brucella melitensis* raises concerns about the potential for this species to cause infectious abortions in endemic areas. Therefore, further research with a larger sample size is necessary to comprehensively assess the involvement of *Brucella* spp. in spontaneous miscarriages and the associated implications for maternal and fetal health.

**Keywords:** *Brucella melitensis*, spontaneous abortion, miscarriage, Iran.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Study of prevalence of *Rhodococcus equi* in horses of Golestan province of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Rhodococcus equi* is a Gram positive bacterial pathogen and one of the most common cause of severe pneumonia in foals less than 6 months of age. The pathogen is ubiquitous in the environment, lives in soil and is present in most areas where horses are kept. this bacterium can cause disease in human beings. The purpose of this study was to evaluate the presence of the genome of VapA of *Rhodococcus equi* in horses of some cities of Golestan province of Iran using PCR method.

### MATERIALS AND METHODS

In the present study, 150 horses of Golestan province were randomly selected and nasal swab samples and epidemiological informations including age, gender and history of disease with a questionnaire were taken. The DNA of the samples was extracted and *Rhodococcus equi* was identified by PCR method and the effect of age, gender and history of disease of horses on the level of contamination was investigated using the Pearson's Chi-square test.

### RESULTS AND DISCUSSION

In the present study, out of a total of 150 examined horses, infection with *Rhodococcus equi* was found in 43 cases (28.7%) of the horses. In this study, the rate of prevalence in horses under 6 months of age was higher than others and the Pearson's Chi-square test showed that it is statistically significant. Mares had a higher prevalence rate than stallions and no significant relationship was observed in the Pearson's Chi-square test. The prevalence rate was higher in horses that had a history of disease and the Pearson's Chi-square test showed that it is statistically significant.

### CONCLUSION

This study showed that the prevalence of this bacterium in Golestan province is significant and requires appropriate programs and acts to control and prevent these diseases.

**Keywords:** *Rhodococcus equi*, Horse, Nasal swab, PCR, Golestan, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Study of prevalence of *Streptococcus equi* subspecies *equi* and *Streptococcus equi* subspecies *zooepidemicus* in horses of Golestan province of Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Streptococcus equi* subspecies *equi* is the cause of strangles disease and is one of the most common and important infectious diseases of equidae. *Streptococcus equi* subspecies *zooepidemicus* is considered part of the flora of the respiratory tract of horses and causes respiratory disease. These two bacteria can also cause diseases in human beings. The purpose of this study was to evaluate the presence of the genome of SodA of *Streptococcus equi* subspecies *zooepidemicus* and SeM of *Streptococcus equi* subspecies *equi* in horses of Golestan province of Iran using PCR method.

#### MATERIALS AND METHODS

In the present study, 150 horses of Golestan province were randomly selected and nasal swab samples and epidemiological informations including age, gender and history of disease with a questionnaire were taken. The DNA of the samples was extracted and these bacteria were identified by PCR method and the effect of age, gender and history of disease of horses on the level of contamination was investigated using the Pearson's Chi-square test.

#### RESULTS AND DISCUSSION

In the present study, out of a total of 150 examined horses, infection with *Streptococcus equi* subspecies *equi* and *Streptococcus equi* subspecies *zooepidemicus* was found in 23 cases and 54 cases of the horses, respectively. In this study, the rate of prevalence of both bacteria in horses with 3-14 years old were higher than others and In both bacteria, Mares had a higher prevalence rate than stallions and also the prevalence rate of both bacteria was higher in horses that had a history of disease.

#### CONCLUSION

this study showed that the prevalence of these bacteria in Golestan province is significant and requires appropriate programs and acts to control and prevent these diseases.

**Keywords:** *Streptococcus equi*, *Streptococcus zooepidemicus*, Horse, Nasal swab, PCR, Iran

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibacterial effect of *Ferula foetida* oleo-gum-resin aqueous extract on some Gram-positive and Gram-negative bacteria

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Antibacterial resistance has increased dramatically worldwide over the past few years and is now recognized as a major challenge in most countries. Therefore, today the use of plants with antimicrobial properties has increased in a large part of the world, especially in developing countries. *Ferula* plant is a member of the *Apiaceae* family, which has more than 170 species and is distributed from Central Asia to West to North Africa. Most species of *Ferula* have a strong odor due to oleoresin. The bioactive compounds of these plants, including sulfur-containing compounds, terpenoids, coumarins, and sesquiterpenes, can have antimicrobial properties. So, the purpose of this study was to investigate the antibacterial effect of the aqueous extract of *Ferula foetida* oleo-gum resin on some Gram-positive and Gram-negative bacteria.

### MATERIALS AND METHODS

*Ferula foetida* oleo-gum resin was collected from Beirut village, Razavi Khorasan. To prepare the aqueous extract of *F. foetida* oleo-gum-resin, 25 grams of plant gum was added to 250 ml of distilled water and shaken for 48 hours at a speed of 1500 rpm, at 37°C. Then the solution was filtered with sterile gauze and filter paper and freeze-dried for 24 hours. Finally, 7.908 g of aqueous extract was obtained and stored in a freezer at -70°C. To evaluate the antibacterial effects of the obtained extract on *Staphylococcus aureus*, *Streptococcus agalactiae*, *Listeria monocytogenes*, *Corynebacterium bovis*, and *Escherichia coli* the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extract were determined using microdilution method in microtiter plates with cation-adjusted Mueller-Hinton broth according to the clinical and laboratory standards institute (CLSI) protocols.

### RESULTS AND DISCUSSION

For *Streptococcus agalactiae*, *Listeria monocytogenes*, and *Corynebacterium bovis*, the MIC of *Ferula foetida* oleo-gum-resin aqueous extract was 4 mg/ml. For *Staphylococcus aureus* and *Escherichia coli*, MICs were determined to be 8 mg/ml and more than 32 mg/ml, respectively. The MBCs of the extract were 32 mg/ml for *Streptococcus agalactiae* and *Listeria monocytogenes* and more than 32 mg/ml for *Staphylococcus aureus*, *Corynebacterium bovis*, and *Escherichia coli*.

### CONCLUSION

The extract is more effective against *Streptococcus agalactiae* and *Listeria monocytogenes*. The bacteriostatic activity of the extract is stronger than its bactericidal activity.

**Keywords:** *Ferula foetida* oleo-gum resin, Antibacterial effect, Bacteria, MIC, MBC

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Seroconversion age of EBV among children under 10 years in Mashhad

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Epstein-Barr virus, also known as Human gammaherpesvirus 4, is one of the eight known viruses belonging to the herpesvirus family. The virus can infiltrate a multitude of cells, including B lymphocytes and epithelial cells. Researchers have conducted research on the age of seroconversion and its correlation to B lymphocyte malignancies in children in various geographical regions because of the significance of the Epstein-Barr virus.

Our community's data shortages on the infection rate of the infection at the primary age of seroconversion spotlight the requirement for further research. This study endeavors to investigate the age of seroconversion for children aged 10 and under in Mashhad.

### MATERIALS AND METHODS

We investigated at Imam Reza, Akbar, and Qaim Hospital, where ethical regulations and protocols were strictly enforced during the sampling procedure. We collected the samples from the leftover serum of children who had routine tests done at the Qaim and Akbar special clinics. For confidentiality, we took 283 serum specimens at random from children no older than 10 years, with their names, ages, and sex codes kept secret. The samples were divided evenly between the two sexes and were subsequently tested using the EBV antibody ELISA test kit.

### RESULTS AND DISCUSSION

The study evaluated 283 children below 10 years of age, with 59.2% being boys, 40.8% girls, and gender information missing for six children. Among them, 29% tested positive for the EBV antibody. We observed the lowest frequency of positive results in the age group with the least amount of antibodies. The age group with the highest frequency of positive results was under six months, with 55.6%, while the age group between 7-10 years recorded the lowest frequency of 6.5%. Statistical analysis revealed a significant difference between age groups, while we observed no significant difference based on gender.

### CONCLUSION

Out of the children examined, 29% had an EBV antibody in their system. Analysis of positive results based on age groups showed the highest frequency in children below six months of age (55.6%) and the lowest frequency in the age group of 7-10 years (6.5%). A significant difference was observed between different age groups, while no significant difference was found based on gender. The study identified the age of seroconversion to be between 3-4 years old.

**Keywords:** Seroconversion, EBV, Elisa

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Frequency study of JC virus in patients with Hodgkin and Non- Hodgkin lymphoma

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

The John Cunningham virus (JCV) is a small, non-enveloped virus with a circular double-stranded genome. It is classified as a pandemic infection and belongs to the *Polyomaviridae* family. JCV can cause a subclinical infection that results in lifelong latency. However, the latent form of the virus can become active in individuals with immune system deficiencies. JCV is known to induce cellular transformation in human B lymphocytes by interacting with various proteins, such as p53, retinoblastoma family proteins, and cell cycle regulators. Recently, researchers have linked JCV to several types of cancer, including Hodgkin's and non-Hodgkin's lymphoma, as well as reactive lymphoma.

The primary aim of this study was to analyze the frequency of the JC virus in paraffin samples got from patients diagnosed with Hodgkin's and non-Hodgkin's lymphomas, as well as reactive lymphoma.

### MATERIALS AND METHODS

For this study, 90 paraffin samples were collected from patients diagnosed with Hodgkin's, non-Hodgkin's, and reactive lymphoma from the pathology department and each group contained 30 samples. the tissue was deparaffinized, and its DNA was separated. The real-time PCR method was employed to amplify the JC virus-specific primer in order to detect the virus DNA. To achieve a higher level of accuracy, a Nested PCR technique was implemented.

### RESULTS AND DISCUSSION

After analyzing the patient characteristics, we observed that the mean age was 33, 50, and 46 in the Hodgkin group, non-Hodgkin group, and reactive group, respectively. Nevertheless, the results of the study indicated that the real-time PCR and nested PCR did not detect any signals in the sample, which demonstrated that there was no JC virus present.

### CONCLUSION

The absence of the JC virus in the sample suggests that the infection caused by the virus alone may not be responsible for the development of lymphomas. It is suggested that JCV can cause cancer through the hit-and-run mechanism, in which the presence of the virus is not necessary. Other molecular factors and mechanisms may also contribute to the occurrence of lymphomas in humans.

**Keywords:** Hodgkin, Non- Hodgkin lymphoma, Reactive lymphoma, JC Virus

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Protectivity of a recombinant protein derived from a 5×Loop3 of Omp34 against *Acinetobacter baumannii* in a murine sepsis model

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Acinetobacter baumannii* is a globally recognized primary cause of infections acquired in hospitals. This bacterium, although considered a low-grade opportunistic pathogen, plays a crucial role in causing various types of infections such as ventilator-associated pneumonia, urinary tract infection, skin and wound infections, bacteremia, and meningitis. In light of the emergence of antibiotic-resistant strains, alternative treatment strategies like recombinant vaccines and specific antibodies have gained importance for combating these infectious bacteria. However, the effectiveness of tested bacterial surface antigens has been limited to providing partial protection. Hence, the development of polyvalent (multi-antigen) vaccines incorporating different antigens has become imperative to achieve a satisfactory level of protection. In this study, the focus is on utilizing two outer membrane proteins, namely construct L3x5 and Omp34, as components of a polyvalent vaccine.

#### MATERIALS AND METHODS

It includes plasmid purification, expression, purification, and injection of construct L3x5 and Omp34 recombinant proteins into BALB/c mice. Both active and passive immunizations were performed. The mice were subsequently challenged with a clinical isolate of *A. baumannii*. The antibody levels in mice were measured using Indirect ELISA. The survival rate of the challenged animals was also determined.

#### RESULTS AND DISCUSSION

ELISA analysis revealed increased antibody production in all immunized groups. However, the combined administration of the L3x5 construct and Omp34 proteins provided superior protection compared to administering each protein individually.

#### CONCLUSION

Polyvalent vaccines containing multiple antigens offer a satisfactory level of protection.

**Keywords:** *Acinetobacter baumannii*., Recombinant protein., construct L3x5, Omp34., Immunogenicity., Vaccine

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigation of anti-bacterial and anti-cancer effects of curcumin ferromagnetic nanocomposite on *Acinetobacter baumannii* isolates and AGS cell line

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Curcumin, a flavonoid from the rhizome of *Curcuma longa*, has anti-bacterial and anti-cancer activities. This study aimed to design and synthesize a magnetic nanocarrier to deliver curcumin to bacterial and cancer cells.

#### MATERIALS AND METHODS

The physicochemical, anti-bacterial, and anti-cancer properties of curcumin ferromagnetic nanocomposite were measured. In addition, clinical isolates of *Acinetobacter baumannii*, gathered from medical and educational facilities in Tehran, Iran, were treated with ciprofloxacin (sub-MICs) alone and/or in combination with curcumin nanocomposites. The cytotoxicity effects of curcumin were studied on AGS cells.

#### RESULTS AND DISCUSSION

The physicochemical analyses have confirmed the synthesis of the proposed structure for curcumin ferromagnetic nanocomposite. Additional measurements have also been taken to determine the particle size and paramagnetic properties. The anti-bacterial property of curcumin in Fe<sub>3</sub>O<sub>4</sub>@SP particles decreased the expression of efflux pump and biofilm formation related genes and increased the porin genes in combination with ciprofloxacin in *A. baumannii*. MTT assay analysis showed that curcumin ferromagnetic nanocomposite inhibited cell proliferation in a dose- and time-dependent manner and had anti-invasive and anti-migrative effects on AGS cells.

#### CONCLUSION

Our results suggested curcumin in ferromagnetic nanocomposite can be used as anti-bacterial and anti-cancer agent.

**Keywords:** *Acinetobacter baumannii*, Biofilm, Curcumin, Fe<sub>3</sub>O<sub>4</sub> nanocarriers, AGS cells

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Recombinant Expression of a Plant-Derived Dimeric Antifungal Peptide (DiSkh-AMP1) Joined by a Flexible Linker in *Escherichia coli* and Evaluation of Its Biological Activity in-Vitro

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

DiSkh-AMP1, a novel dimeric antifungal peptide contained 65 amino acid residues was recombinant produced by a flexible linker to improve the antifungal activity of native Skh-AMP1 isolated from *Satureja khuzistanica* leaves.

### MATERIALS AND METHODS

To discover a new system for expressing DiSkh-AMP1 in *Escherichia coli* BL21, the DiSkh-AMP1 gene was synthesized and inserted into pET28a vector in which fusion peptide (3.5 kDa) was used as a fusion partner and an N-terminal 6-His as an affinity tag. The fusion/DiSkh-AMP1 peptide expression was induced with 1 mM isopropyl-thio-galactoside (IPTG) for 4 h at 37 °C. The recombinant fusion/DiSkh-AMP1 was then purified by affinity chromatography, digested with cyanogen bromide and isolated from the fusion peptide by reverse-phase high-performance liquid chromatography.

### RESULTS AND DISCUSSION

The final yield of DiSkh-AMP1 was 0.95 mg/L after cleavage with cyanogen bromide. DiSkh-AMP1 strongly inhibited the growth of *Candida albicans* (ATCC 10231) and *Aspergillus fumigatus* (Af 29) by MIC values 6.5 and 6.86  $\mu$ M respectively. It showed fungicidal activity against *Candida albicans* (ATCC 10231) and *Aspergillus fumigatus* (Af 29) with MFC values 13.00 and 13.72  $\mu$ M respectively. DiSkh-AMP1 had a negligible hemolytic activity of 4.1% for human red blood cells.

### CONCLUSION

Taken together, our results demonstrated that DiSkh-AMP1 with improved antifungal activity and suitable yield in expression system could be considered as a potential candidate to be a novel antifungal drug against life-threatening fungal infections.

**Keywords:** Skh-AMP1 · Dimeric antifungal peptide, *Candida albicans*, *Aspergillus* species, Flexible linker, Recombinant expression, *Escherichia coli*.

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# Design, Dimerization, and Recombinant Expression of a Plant-Derived Dimeric Antifungal Peptide (DiMCh-AMP1) in *Escherichia coli* and Evaluation of Its Biological Activity in Vitro

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Fungal species resistant to current antifungal agents are considered as a serious threat to human health, the dilemma that has dragged attentions toward other sources of antifungals such as antimicrobial peptides (AMPs).

### MATERIALS AND METHODS

In order to improve biological activity of a recently described antifungal peptide MCh-AMP1 from *Matricaria chamomilla* flowers, MCh-AMP1 dimer (DiMCh-AMP1), containing 61 amino acid residues connected by flexible linker (GPDGSGPDESGPDES), was designed and expressed in *Escherichia coli*, and its structure was analyzed using bioinformatics tools. DiMCh-AMP1 synthetic gene was cloned into pET-28a expression vector, which was then used to transform *E. coli* BL21 (DE3) strain. His-tag purification was achieved using metal-chelate affinity chromatography. Because there is no methionine residue in the DiMCh-AMP1 sequence, cyanogen bromide was successfully used to separate the target product from the tag. Reverse-phase high-performance liquid chromatography was used as the final step of purification.

### RESULTS AND DISCUSSION

Results showed that recombinant peptide was produced in considerable amounts (0.9 mg/L) with improved antifungal activity toward both yeasts and molds compared to its monomeric counterpart. The minimum inhibition concentration and minimum fungicidal concentration values of DiMCh-AMP1 against *Candida* and *Aspergillus* species were reported in the range of 1.67–6.66 μM and 3.33–26.64 μM, respectively.

### CONCLUSION

Our results showed that while antifungal activity of dimerized peptide was improved considerably, its cytotoxicity was decreased, implying that DiMCh-AMP1 could be a potential candidate to design an effective antifungal agent against pathogenic yeasts and molds.

**Keywords:** Antifungal Activity, Cytotoxicity, Antimicrobial Peptide, *Aspergillus*, *Candida*, Bacterial Expression System

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# Development of a differential Multiplex PCR assay for *Chlamydia abortus*, *Brucella spp.* & *Campylobacter fetus*

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Abortion is one of the most critical factors affecting lambing rates. *Chlamydia abortus*, *Brucella spp.* and *Campylobacter fetus* are main pathogens because of the potential impact on veterinary and human health. Accurate, rapid, and specific methods are required to identify pathogens for controlling bacteria causing abortion in sheep.

### MATERIALS AND METHODS

Multiplex PCR method was optimized for identification of bacterial infections caused by *Chlamydia abortus*, *Brucella spp.*, *Campylobacter fetus*. The sensitivity and specificity of the test were evaluated. The number of 57 samples of aborted sheep fetuses along with the placenta sent to the veterinary laboratory of North Khorasan province in 2021 was examined. The expected sizes of amplicons were 222 bp for *Brucella spp.* (Baily et al., 1992), 479 bp for *Chlamydia abortus* (DeGraves et al., 2003) and 359 bp for *Campylobacter fetus* (Yamazaki et al., 2007).

### RESULTS AND DISCUSSION

In this study, 57 samples were analyzed by single and multiplex PCR method. *Chlamydia abortus* was detected in 46 samples (80%) that simultaneous presence of *Chlamydia abortus* and *Brucella spp.* was confirmed that in one samples. *Brucella spp.* alone was observed in 5 samples, Therefore, in total, 6 samples (10%) were infected with *Brucella spp.*, *Campylobacter fetus* was observed in 3 sample (5%).

### CONCLUSION

This study confirmed the high prevalence of *C. abortus*, *Brucella spp.* and *Campylobacter fetus* among sheep and goat flocks in Iran. Diagnosis of infection by mPCR helps us to use appropriate antimicrobial agents to treat and control the disease. This technique is helpful in epidemiological studies for determinant focality of diseases and prevention of outbreaks. Multiplex PCR is a useful, inexpensive, accurate, and rapid method for identifying bacterial agents and more samples can be identified by this assay in a shorter period of time.

**Keywords:** Multiplex PCR, *Chlamydia abortus*, *Brucella*, *Campylobacter fetus*

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### Prevalence, antibiotic resistance and frequency of virulence factors in *Pseudomonas aeruginosa* strains isolated from meat in Isfahan

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Strains of *Pseudomonas aeruginosa* are thought to be the primary contributors to food deterioration and, sporadically, foodborne illnesses. The goal of the current investigation was to assess the distribution of virulence factors and patterns of antibiotic resistance in *Pseudomonas aeruginosa* strains isolated from meat.

#### METHOD AND MATERIALS

150 samples of frozen meat in total were collected. Using traditional microbial culture, samples were examined for *Pseudomonas aeruginosa* contamination. Using the disk diffusion method, the pattern of antibiotic resistance of *Pseudomonas aeruginosa* isolates was assessed. *Pseudomonas aeruginosa* isolates' genomic DNA was extracted and the polymerase chain reaction was used to determine how frequently virulence factors were present.

#### RESULTS AND DISCUSSION

*Pseudomonas aeruginosa* was found to be infected in 23 out of 150 meat samples (15.3%). *Pseudomonas aeruginosa* also was present in raw and curled meat samples in amounts of 6.4 and 19.7%, respectively. *Pseudomonas aeruginosa* prevalence varied between samples of raw and frozen beef in a statistically significant way ( $P < 0.05$ ). Antibiotic resistance to ampicillin (98%), penicillin (89.6%), and tetracycline (78.3%) was highest in detected isolates of *Pseudomonas aeruginosa*. Antibiotic resistance was less common than in other cases for imipenem (7.2%) and trimethoprim (12.9%). The most often found virulence factors were the *exoU* (67.3%) and *exoT* (23.8%) genes.

#### CONCLUSION

Further research is required to confirm the role of the virulence factors and antibiotic resistance in *Pseudomonas aeruginosa* strains isolated from raw and frozen meat.

**Keywords:** *Pseudomonas aeruginosa*, Meat, Antibiotic resistance, Virulence factors.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of contamination of dairy products with *Listeria monocytogenes* and determination of their antibiotic resistance

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Listeria monocytogenes* is a type of pathogenic bacteria that causes listeriosis infection. One of the most significant infections related to milk and milk products. *Listeria monocytogenes* is the major species that causes listeriosis in people and animals which can spread by contaminated food.

### METHOD AND MATERIALS

For this purpose, 100 samples of raw milk (70 samples), local butter (13 samples) and cheese (17 samples) were selected and collected to identify samples suspected of *Listeria monocytogenes*. The desired samples were taken to the laboratory for diagnosis by observing the temperature cycle. The approved samples were selected to evaluate the antimicrobial sensitivity of *Listeria monocytogenes* isolates by disc diffusion method and using ampicillin, gentamicin, erythromycin, chloramphenicol, tetracycline, ciprofloxacin antibiotics.

### RESULTS AND DISCUSSION

Of the total samples of *Listeria monocytogenes* isolated from dairy products, 35% of raw milk products, 15% of local butter samples, and 24% of cheese samples were infected with *Listeria monocytogenes*. The results of the antibiotic resistance of dairy samples showed that the resistance to ampicillin was 66.4% and tetracycline was 54.3%. The highest level of *Listeria* resistance in butter samples was to erythromycin (69.6%) and in cheese samples to ampicillin (71.2%). The average results obtained in the antibiogram test in samples resistant to *Listeria monocytogenes* were analyzed with 95% confidence and one-way analysis of variance.

### CONCLUSION

Based on the results of this study, the risk of contamination of raw dairy products with *Listeria monocytogenes* is increasing and this contamination is more in samples of raw milk and local butter than in other products.

**Keywords** *Listeria monocytogenes*, raw Milk, dairy products, cheese, butter samples.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of the effect of Genistein, *Rosmarinus officinalis*, On EGFR protein by molecular docking method

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Estrogen receptor is usually used as the first treatment for patients with breast cancer. EGFR targeted therapy for breast cancer has promising effects for patients with breast cancer. EGFR, related pathways with EGFR, may contribute to some aspects of the biological behavior of breast cancer. Therefore, blocking the receptor can lead to the suppression of breast cancer. The aim of this study is to determine which of the two drugs genistein, *Rosmarinus officinalis* has a better effect on the EGFR protein.

### MATERIALS AND METHODS

In this study, the Pub Chim site at pubchem.ncbi.nlm.nih.gov, Drugbank www.drugbank.com and www.uniprot.org was used. ViewerLite software, AutoDockTools-1.5.6, Chimera 1.15 and PyRx was also used. In this article, we first saved Genistein and *Rosmarinus officinalis* from a site in PDF format. specifications of Genistein: Molecular formula: C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>

Genistein is an isoflavone originally isolated from the Dyer's broom plant. Widely distributed in the Fabaceae family. Investigating the mechanisms of anticancer activity of many pathways, including induction of cell proliferation, suppression of tyrosine kinases, regulation of signaling, modulation of epigenetic activities, cell cycle arrest, and Akt and MEK signaling pathways. specifications of *Rosmarinus officinalis*: Molecular formula: C<sub>20</sub>H<sub>28</sub>O<sub>4</sub>

### RESULTS AND DISCUSSION

This systematic review aimed to evaluate the potential anti-inflammatory effect of *Rosmarinus officinalis* in clinical models of inflammation in vivo. Edited the target protein using Chimera 1.15 software. EGFR protein has four chains. In this study, we only used chain A for better matching, the other chains were removed. Also, using this software, water molecules were removed from the protein and hydrogen molecules were added to its structure. Then, using PyRx software, we started the molecular connection.

To choose the right connection place was as follows:

resolution: 6.05

center\_x = -13.524

center\_y = -165.57

center\_z = -29.8362

size\_x = -13.524

size\_y = -165.57

size\_z = -29.8362

### CONCLUSION

According to docking results, it was observed that *Rosmarinus officinalis* can be a suitable drug for binding to EGFR protein

**Keywords:** bioinformatics, molecular docking, EGFR, genistein, *Rosmarinus officinalis*

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# Phenotypic and genotypic investigation of fluoroquinolones resistance in *Klebsiella pneumoniae* clinical isolates in Bushehr province, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The emergence of antimicrobial resistance is one of the main global health problems, and the alarming spread of microorganisms resistant to antimicrobial drugs is considered a serious threat. The aim of this study was to investigate the phenotypic and genotypic fluoroquinolones resistance in *Klebsiella pneumoniae* clinical isolates in Bushehr province, Iran.

### MATERIALS AND METHODS

A total of 215 of *K. pneumoniae* isolates collected from six hospitals in Bushehr province during 2017-2019 were used. Antimicrobial susceptibility test for fluoroquinolones was determined by disk diffusion and E-test methods. The presence of plasmid mediated quinolone resistance (PMQR) genes including *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *OqxA*, *aac(6)-Ib-cr*, and *qepA* was detected by PCR method. In addition, mutations in *gyrA* and *parC* genes in ciprofloxacin-resistant *K. pneumoniae* isolates were identified by PCR and sequencing.

### RESULTS AND DISCUSSION

Out of 215 *K. pneumoniae* isolates, 49 isolates (22.7%) were resistant to ciprofloxacin in disc diffusion method. Among 49 ciprofloxacin-resistant isolates, 40 isolates were resistant by E-test. MIC to ciprofloxacin ranged from 4 µg/ml to ≥32 µg/ml. In addition, PCR results revealed that among 40 ciprofloxacin-resistant isolates, 13 isolates (32.5%), 7 isolates (17.5%), 40 isolates (100%) and, 25 isolates (62.5%) harbored *qnrB*, *qnrS*, *oqxA* and *aac(6)-Ib-cr* genes, respectively. In this study *qepA*, *qnrC*, *qnrD* and *qnrA* genes were not found. Mutation analysis of *gyrA* and *parC* genes showed that out of 40 ciprofloxacin-resistant isolates, 35 (87.5%) and 34 isolates (85%) had mutations in *gyrA* and *parC* gene, respectively. The most mutations were observed in codon 80 of *gyrA* and codon 83 of *parC* gene.

### CONCLUSION

Despite the presence of PMQR genes, mutations in the chromosomal genes play a major role in the occurrence of fluoroquinolone resistance among *K. pneumoniae* clinical isolates in Bushehr province.

**Keywords:** *Klebsiella pneumoniae*, Quinolone, Mutation, *parC*, *gyrA*, PMQR

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# Genotypic and phenotypic antibiotic resistance in *Escherichia coli* isolates obtained from intestine of retail rainbow trout (*Oncorhynchus mykiss*) in Kerman, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Antibiotics are employed worldwide to treat diseases of humans and other animals. The uncontrolled use of antibiotics increases the emergence of resistant bacteria and makes it difficult to treat the infections.

### MATERIALS AND METHODS

One hundred and sixty-six rainbow trout fish were randomly collected from different retail stores in Kerman City, southeast of Iran. Intestine samples were obtained from each fish using a swab. After the isolation and biochemical confirmation process, *Escherichia coli* (*E. coli*) isolates were screened for antimicrobial resistance (AMR) and phenotypes.

### RESULTS AND DISCUSSION

*E. coli* strains were isolated from 8.60% of the swabs. Among the isolated strains, the most prevalent resistance genes were *dhfrI* (40%), followed by *bla<sub>CTX-M</sub>* (20%), *sulI* (20%), *tetB* (20%), *sul2* (20%), and other resistance genes (*tetA*, *dhfrV*, *qnrA*, *catI*, *bla<sub>TEM</sub>*, *bla<sub>SHV</sub>*, *bla<sub>OXA</sub>*) did not exhibit any prevalence. The findings revealed high resistance rates to antibiotics such as erythromycin (80%), ciprofloxacin (60%), amoxicillin (60%), trimethoprim (60%), Oxytetracycline (60%), nitrofurantoin (60%), flumequine (60%), tetracycline (40%), chloramphenicol (40%), florfenicol (40%), trimethoprim-sulphamethoxazole (20%), and cefotaxime (20%). Additionally, 40% of the isolated *E. coli* strains tested positive for extended-spectrum beta-lactamases (ESBL).

### CONCLUSION

These findings indicate that retail rainbow trout can serve as passive carriers of multidrug-resistant *E. coli* strains, highlighting the importance of adopting proper hygiene practices, managing water quality, and practicing responsible antibiotic use in fish farming to minimize the spread of antibiotic-resistant bacteria.

**Keywords:** Genotypic, phenotypic, *Escherichia coli*, rainbow trout, intestine.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of physical, chemical, and physicochemical treatments for overcoming the inhibitory effect of heparin on DNA amplification in realtime-PCR

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Heparin is a highly sulfated glycosaminoglycan used as an injectable anticoagulant. Heparin acts indirectly at multiple sites in both internal and external blood coagulation pathways. Blood is a common source for tracking DNA samples for various microbial and non-microbial tests to prevent coagulation from EDTA-containing tubes. mg1 is used per milliliter.

Heparin is also a common anticoagulant, but because it has a strong inhibitory effect on PCR, it is not used for samples to be DNA tracked. In all dilutions, heparin inhibits virus replication in the real-time PCR test

### MATERIALS AND METHODS

In this study, we intend to investigate different ways to remove the inhibitory effect of heparin in PCR. There are three physical, chemical, and enzymatic methods to remove the effect of heparin, which we used chemical and physical methods in this experiment. First, blood samples were taken from a dog and poured into tubes containing EDTA and heparin. Then, 7 vaccines were used as a source of virus and finally, DNA extraction was performed by phenol-chloroform method.

### RESULTS AND DISCUSSION

Two methods were adopted for chemical treatment. The first method was to use an ascorbic acid-oxygenated water combination. In this method, different concentrations of ascorbic acid (20, 10, 5, 1, and 30 mM) and temperatures (35, 25, 15, and 45) during ultrasonic operation and ultrasonic intensity (16, 11, 7, 6, and 21W / ml) during Ultrasonic surgery was considered. The second method was the use of nitrous acid at pH 3 and 1. 5. For the heat treatment of heparin, Ben Marie 65 ° and 85 ° for 2 hours and 121 ° autoclave for half, 1, and 2 hours were used.

### CONCLUSION

At the end of the analysis of the results in different methods of heparin inhibitory effects, it was found that the best method among the methods performed is the use of 85 ° C and 85 ° C on extracted genome for 2 hours. After that, heat treatment at 85 ° C for 2 hours on blood samples before extraction and finally nitrous acid (pH = 3) treatment.

**Keywords:** Elimination of heparin inhibition, Realtime-PCR, Temperature, H<sub>2</sub>O<sub>2</sub>/ascorbic acid, sonication, Nitrous acid

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Development of a multiplex PCR assay for the detection of *ISAbal*, *AAC* and *QNR* genes in *Acinetobacter baumannii* isolates in Tehran city, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*ISAbal* are islands of antibiotic resistance genes, which are known as global antibiotic resistance islands. Integron acquisition is one of the important factors of multi-resistance in Gram-negative microorganisms. The simultaneous presence of integron along with antibiotic resistance genes indicate the emergency status of bacteria carrying antibiotic resistance genes. *QNR* are antibiotic resistance genes that play a role in antibiotic resistance to quinolones and fluoroquinolones. *AAC* are resistance genes to aminoglycosides that include several subclasses. In recent years there has been an expanded frequency of MDR, XDR and even PDR *A. baumannii* infections, which has been creating intense issues among patients admitted to hospitals. So, the use of rapid diagnostic methods such as Multiplex PCR increases simultaneous detection of drug resistance genes and help physicians to select the proper antibiotic. Therefore the current study is the first study that designed a multiplex PCR method for the concurrent detection of IQA genes (*ISAbal*, *AAC*, *QNR*) from *Acinetobacter baumannii* strains in Tehran city in Iran.

### MATERIALS AND METHODS

A set of 100 clinical *Acinetobacter baumannii* strains have been gathered from burn samples of patients which were hospitalized in Motahhari hospital in Tehran, Iran. Antibiotic susceptibility was conducted by Kirby–Bauer disc diffusion test. Nine antibiotic discs including ampicillin (10µg), ceftazidime (30µg), cefotaxime (30µg), imipenem (10µg), ciprofloxacin (5µg), gentamicin (10µg), amikacin (30µg), cefepime (30µg) and colistin (10µg) were used to assess Multidrug-resistant (MDR) and Extensive drug-resistance (XDR) isolates. Multiplex PCR were characterized for the simultaneous diagnosis of *ISAbal*, *AAC* and *QNR* genes.

### RESULTS AND DISCUSSION

This study showed most of the strains were XDR and the highest sensitivity was for Colistin. Results of multiplex PCR showed that the prevalence of *ISAbal*, *QNR* and *AAC* genes in clinical isolates of *A. baumannii* were 42/100, 0/100, 0/100, respectively

### CONCLUSION

The current study showed that *ISAbal* gen was prevalent among clinical strains of *Acinetobacter baumannii* while no amplification of *QNR* and *AAC* genes was seen and the increase in resistance to common antibiotics among hospital strains of *Acinetobacter baumannii* is an alarm for the medical community and researchers. Multiplex PCR can be a reliable and sensitive technique for rapid detection of resistance genes in clinical strains.

**Keywords:** Multiplex PCR, *Acinetobacter baumannii*, *ISAbal*, *AAC*, *QNR* genes

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# Antibiotic resistance patterns of *Pseudomonas* spp. isolated from transplant and non-transplant patients in Abu-Ali-Sina hospital of Shiraz

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Antimicrobial resistance, a growing global problem, is recognized as one of the main causes of mortality around the world. In transplant recipients, multidrug-resistant pathogens are associated with significant complications despite advanced surgical techniques, the development of immunosuppressive drugs, antibiotics, vaccines, and infection control measures. Therefore, the aim of this study was to investigate the antibiotic resistance profile of *Pseudomonas* spp. isolated from transplant and non-transplant patients in Abu-Ali-Sina hospital of Shiraz.

### MATERIALS AND METHODS

In a cross-sectional study, *Pseudomonas* spp. were isolated from different clinical samples during a period of 6 months. The strains were identified by standard microbiological tests. Antimicrobial sensitivity test was performed using the Kirby-Bauer disk diffusion method, according to the CLSI guideline. Finally, the data were analyzed using the Statistical Package for the Social Sciences software (SPSS, version 25.0).

### RESULTS AND DISCUSSION

In total, during this study, 49 clinical isolates of *Pseudomonas* spp. were isolated from transplant 21(42/9%) and non-transplant 28(57/1%) patients. In transplant patients, the most isolation was related to liver transplant 11(22/4%), followed by kidney transplant 9(18/4%). The highest and lowest bacterial isolates in both groups were obtained from sputum 15(30/6%), urine 13(26/5%) and blood 5(10/2%) samples, respectively. In non-transplant patients, the highest resistance rate was related to trimethoprim/sulfamethoxazole(92/9%), doxycycline(89/3%), levofloxacin(60/7%), ciprofloxacin(57/1%), meropenem(53/6%), ceftazidime(50%) and the least resistance was related to colistin(7/1%) and piperacillin-tazobactam(35/7%). In transplant patients, the highest resistance rate was related to trimethoprim/sulfamethoxazole(100%), doxycycline(95/2%), ciprofloxacin(76/2%), levofloxacin(76/2%), meropenem(76/2%), piperacillin-tazobactam(71/4%), gentamicin (71/4%), ceftazidime (66/7%) and the least resistance was related to colistin(0%).

### CONCLUSION

Considering that strains isolated from transplant patients had higher resistance to the investigated antibiotics, thus, applying the best policies and strategies to reduce antibiotic resistance and strengthen rational antibiotic treatment is essential in these patients.

**Keywords:** Antimicrobial resistance, *Pseudomonas* spp., Transplant patients

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Effect of Phage-antibiotic combination therapy against clinical multidrug-resistant *Acinetobacter baumannii* isolates

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

*Acinetobacter baumannii* is a major cause of nosocomial infections globally, and the increasing prevalence of multidrug-resistant strains of this bacterium has become a significant public health concern. To combat the rise in drug resistance, alternative approaches such as phage therapy have garnered considerable attention.

#### MATERIALS AND METHODS

Phage isolation and enrichment were performed using hospital wastewater samples collected from the same hospital. Phages specific to *A. baumannii* were isolated and purified using filtration methods and a double-agar overlay technique. Various experiments such as the efficiency of plating determination, the optimal multiplicity of infection (MOI), morphology analysis by transmission electron microscopy (TEM), one-step growth curve analysis, minimum inhibitory concentration (MIC) determination, and time-kill curve analysis for assessing combination therapy with antibiotics were conducted to further characterize bacteriophage vB\_AbM\_SA1.

#### RESULTS AND DISCUSSION

Bacteriophages belonging to the myoviridae family have been isolated in this study. It has a short latent period of only 10 minutes and a burst size of 20 PFU/ml. Phage-antibiotic synergy (PAS) is discussed as a promising approach against multidrug-resistant bacteria. The study observed a decrease in minimum inhibitory concentration (MIC) when combining bacteriophage with meropenem, ampicillin-sulbactam, or colistin antibiotics.

Time-kill tests demonstrated varying synergistic effects between phage and different antibiotic concentrations against specific isolates of *A.baumannii*.

#### CONCLUSION

In conclusion, the emergence of antibiotic-resistant strains of *A. baumannii* has led to the exploration of alternative therapies, such as phage therapy. The combination of meropenem, ampicillin-sulbactam, and colistin and phage therapy has been found to be more effective in reducing the bacterial load of *A. baumannii* in vitro than either treatment alone. The use of combination therapy may have significant implications in the treatment of *A. baumannii* infections and warrants further investigation.

**Keywords:** *Acinetobacter baumannii*, Bacteriophage, Drug Resistance, Time-kill, colistin, ampicillin-sulbactam, meropenem, synergy, synergistic effect

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# Identification of the role of HPV in HNSCC tumors and margin healthy tissues and its association with demographic and clinicopathologic characteristics

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Human papillomavirus (HPV) is a DNA virus in the papillomavirus family that is transmitted primarily through sexual contact. More than 200 different genotypes of HPV have been identified, and some of these genotypes are specifically implicated in the occurrence of head and neck cancer. As HPV is known to be an important etiological factor in the development of head and neck squamous cell carcinoma (HNSCC). The aim of this study was to investigate the presence of HPV in both HNSCC tumor tissues and adjacent margin healthy tissues and to explore its association with demographic and clinicopathological characteristics.

### MATERIALS AND METHODS

This study was performed with a total of 62 patients diagnosed with HNSCC. Tumor tissues and corresponding margin healthy tissues were collected from each patient. HPV detection was performed by polymerase chain reaction (PCR) for MY09 and MY11 genes. Demographic and clinicopathological data, including age, gender, smoking status, alcohol consumption, and tumor stage, were obtained from medical records.

### RESULTS AND DISCUSSION

The results showed that HPV was detected in 12.90% of HNSCC tumor tissues and 4.83% of margin healthy tissues, which was significant ( $P=0.01$ ). In the relationship between HPV status and demographic and clinical pathology characteristics, it was found that there was a significant association between HPV detection in tumor tissues and lymph node involvement ( $P=0.03$ ) and histological grade ( $P=0.003$ ). The presence of positive HPV in tumor tissues showed a statistically significant association with older age ( $p < 0.05$ ) and advanced tumor stage ( $p < 0.001$ ). However, there was no significant association between HPV detection in tumor tissues and smoking, alcohol, and opium consumption.

### CONCLUSION

This study demonstrates the presence of HPV in both HNSCC tumor tissues and adjacent margin healthy tissues. The higher prevalence of HPV in HNSCC tumor tissue and its relationship with demographic and clinical pathology characteristics demonstrates the importance of HPV testing in the diagnosis and management of HNSCC. These findings contribute to a better understanding of the role of HPV in HNSCC and may have implications for personalized treatment strategies in the future.

**Keywords:** Grade, head and neck squamous cell carcinoma, human papillomavirus, stage.

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# Studying the presence and abundance of pathogenic genes involved in hyphae production in *Candida albicans* isolated from patients with MS

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Multiple sclerosis (MS) is a chronic debilitating disease of the nervous system. Studies have shown that this disease may be associated with weakness in the immune system. On the other hand, today we know that opportunistic infections affect people who have weakness or defects in the immune system. In addition, one of the most important microorganisms that play a role in causing opportunistic infections is *Candida albicans*, which causes opportunistic fungal infections in patients with MS. The objective of this study was to measure the frequency of some selected genes involved in pathogenesis such as *Hwp1*, *Als1* and *Als3* in the production of fungal filaments in *C. albicans*.

### MATERIALS AND METHODS

Random sampling was done from patients with MS and healthy cases. Yeast isolates were identified by morphological and biochemical methods. Then, the antifungal susceptibility test was determined via disk diffusion assay following CLSI M27-A3 protocol. Subsequently, the frequency of the selected genes was done using the conventional PCR method.

### RESULTS AND DISCUSSION

Twenty seven isolates of *C. albicans* were isolated and identified from the studied subjects. The results showed that 92.6% (25 isolates) and 7.4% (2 isolates) were sensitive and resistant to fluconazole, respectively. Also, the frequency of *Hwp1*, *Als1* and *Als3* genes was 81.5%, 47.0% and 40.7%, respectively. This result showed that the abundance of genes involved in *Candida* filament production is significant in the studied patients. On the other hand, it has been found that the frequency of *Hwp1* gene was significantly different from the other two genes between healthy cases and patients.

### CONCLUSION

The results of the present study have shown a high frequency of studied genes in clinical isolates. Therefore, it can be concluded that the presence of *Candida* in the studied patients is not just a normal flora, but by decreasing the immune system of these people, *Candida* has acted pathogenically through the production of filaments. In addition, these results indicate the fact that infection with *C. albicans* may be associated with a possible increase in the severity of MS. Therefore, it is suggested that in future studies, fungi should be considered as a possible risk factor for increasing MS.

**Keywords:** *Candida albicans*; Fluconazole; Multiple Sclerosis

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# Inhibitory effect of menthol and thymol on MexCD-OprJ efflux pump of *Pseudomonas aeruginosa* under In-vitro and In-silico conditions

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Many studies have documented the inhibitory influence of bioactive compounds of herbal origins on efflux pumps in antibiotic-resistant bacteria. Here, the efficacy of menthol and thymol, as two natural derivatives, was investigated against four strains of *Pseudomonas aeruginosa* using in-silico molecular docking and ethidium bromide-agar cartwheel methods.

### MATERIALS AND METHODS

Serial two-fold dilutions were prepared in tubes assigned to each herbal compound (menthol and thymol) and bacterial strain. The sub-MIC values were calculated after culturing the bacteria on Mueller-Hinton agar (MHA). Subsequently, the cartwheel test (for the strains, with and without exposure to menthol and thymol) was carried out on MHA media containing ethidium bromide and then the plates were observed in a UV transilluminator. Turning to the in-silico study, the three-dimensional structures of menthol and thymol as the ligands and MexCD-OprJ protein as the pump receptor were obtained from PubChem and protein databases, correspondingly, and were evaluated in molecular docking using Autodock (version 4.1).

### RESULTS AND DISCUSSION

The cartwheel method demonstrated reduced pump activity in all strains grown under sub-MIC values of the bioactive compounds, which displayed the red color of ethidium bromide under UV light. It was found that menthol has a higher inhibitory potential and shows greater binding energy ( $\Delta G = -5.81$  Kcal.mol<sup>-1</sup>) with MexCD-OprJ protein compared to thymol ( $\Delta G = -5.63$  Kcal.mol<sup>-1</sup>). Menthol only interacts with amino acids serine B:57 and glutamine B:60, while thymol is able to interact with glycine A:303, serine A:305, threonine A:304, serine A: 319, tryptophan A:320, glutamine A:111, serine A:301, threonine A:88, alanine A:86, alanine A:115, glycine A:113, leucine A:114, phenylalanine A:302, and valine A: 112.

### CONCLUSION

These results suggest that menthol and thymol can be proposed as potential inhibitory agents against various strains of *Pseudomonas aeruginosa*.

**Keywords:** Cartwheel method, Menthol, MexCD-OprJ Efflux pump, Molecular docking, *Pseudomonas aeruginosa*, Thymol

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Menthol and thymol reduce the expression level of the *OprJ* gene in *Pseudomonas aeruginosa*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Antibiotic efflux is one of the most important mechanisms of bacterial resistance to antibiotics. Studies have shown that various plant-derived compounds are able to deactivate efflux activity. Here, the effect of menthol and thymol as bioactive compounds on the expression of the MexCD-OprJ efflux pump encoding gene in different isolates of *Pseudomonas aeruginosa* was assessed through molecular methods.

### MATERIALS AND METHODS

The genomic DNAs of bacterial isolates were extracted using a DNA Extraction Kit (SinaClon, Iran) to screen them for the presence of the OprJ gene. To analyze the expression of the pump gene, real-time PCR (for the ones exposed to bioactive compounds, and controls without bioactive compounds) was carried out. Bacterial isolates of *P. aeruginosa* were cultured in broth media under sub-MIC values of menthol and thymol. The controls were prepared without exposure to bioactive compounds. The total RNAs of the overnight cultures were extracted using an RNA Extraction Kit (SinaClon, Iran). The cDNAs were synthesized from mRNAs using a cDNA Synthesis Kit (SinaClon, Iran), and then real-time PCR was conducted according to the manufacturer's protocol. Data were analyzed by student's t-test using GraphPad Prism 8.

### RESULTS AND DISCUSSION

The results of the real-time PCR revealed that both bioactive compounds managed to significantly decrease ( $p < 0.05$ ) the expression of the OprJ gene.

### CONCLUSION

Given the molecular evidence provided in the current research, menthol and thymol can serve as inhibitory candidates of *P. aeruginosa*'s MexCD-OprJ efflux pump to pave the path for antibiotic treatment.

**Keywords:** Efflux pump, Menthol, MexCD-OprJ, OprJ gene, *Pseudomonas aeruginosa*, Thymol

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Comparative Proteom Analysis of pathogenic and non-pathogenic *Neisseria* Species for the Identification of Immuno-Reactive Antigens

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Neisseria meningitidis* is one of the major causative agents of bacterial meningitis in humans, especially among young children with high morbidity and mortality rates worldwide. Any delays in diagnosis and treatment of meningococcal disease can decrease the chance of survival. Unfortunately, there is still no universal vaccine against serogroup B strains. Antigenic similarities between *N. lactamica* as commensal species and *N. meningitidis* serogroup B (NmB) have been considered to develop an effective vaccine based on their common proteins to prevent life-threatening bacterial meningitis.

### MATERIALS AND METHODS

The whole proteome profiles of *N. lactamica* strains and a reference strain of the NmB were compared. Lysates from bacterial strains were resolved by two-dimensional gel electrophoresis (2-ED), followed by Coomassie Brilliant Blue staining. Some of the matched protein spots were excised from the gel and subjected to matrix-assisted laser desorption/ionization-tandem time-of-flight mass spectrometry (MALDI-TOF/TOF MS) analysis. In the present study, aiming to find a set of common antigenic proteins between NmB and *N. lactamica* strains, 2-DE-immunoblot of any studied bacterial strains was probed with sera from immunized mice corresponds to the same bacterial strains.

### RESULTS AND DISCUSSION

The analysis of Coomassie-stained gels using ImageMaster 2D Platinum software identified approximately 800 protein spots. In addition, the results of 2-DE gels showed that *N. lactamica* strains have approximately a proteome profile similar to each other and slightly different from NmB. MALDI-TOF/TOF MS identified 47 common proteins between two species including membrane and cytoplasmic proteins. Using 2-DE-immunoblot analyses, seven protein spots were identified as common immuno-reactive proteins between the two studied species. Some of them such as outer membrane protein P1, competence lipoprotein, Thioredoxin reductase, and enoyl-ACP reductase are novel and can be recommended as novel vaccine candidates.

### CONCLUSION

These results underscore the usefulness of proteome and immunoproteome analysis in successful identification of the common proteins and common immune-reactive proteins between *N. lactamica* strains and NmB as well. Further investigations are warranted to the possible applications of the present data in recommendation of the novel identified immune-reactive proteins as vaccine candidates against NmB.

**Keywords:** *Neisseria meningitidis* serogroup B; *Neisseria lactamica*; Two-dimensional gel electrophoresis; Mass Spectrometry; children

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Thymol and menthol as two natural bioactive compounds reduce antibiotic resistance of *Pseudomonas aeruginosa*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa*, which is the main cause of nosocomial infections, is resistant to various antibiotics. Hence, this study investigated the efficacy of menthol and thymol, as two natural derivatives, on the antibiotic resistance of *Pseudomonas aeruginosa*.

### MATERIALS AND METHODS

The antibiotic susceptibility of the strains was evaluated using the disk diffusion method. Five standard antibiotic disks, namely Ciprofloxacin (CP5), Levofloxacin (LEV5), Imipenem (IPM10), Ceftazidime (CAZ30), and Aztreonam (AZT30) were used in the test. To check the effectiveness of the antibiotics without bioactive compounds (menthol and thymol), bacterial suspensions ( $1.5 \times 10^8$  CFU.mL<sup>-1</sup>) were streaked out on plates containing Mueller-Hinton Agar, followed by placing the antibiotic disks on the culture media. The sub-MIC values of thymol ( $\bar{x} = 12.79$  mg.mL<sup>-1</sup>) and menthol ( $\bar{x} = 9.37$  mg.mL<sup>-1</sup>) for each bacterial strain were obtained and then the susceptibility test in the presence of the bioactive compounds was carried out following the same procedure taken in the test without the compounds. The diameter of the inhibitory zone (DIZ) was measured after the incubation and the resistance profiles were determined according to the CLSI guidelines.

### RESULTS AND DISCUSSION

The antibiotics were not able to inhibit the growth of the bacterial strains in the antibiogram test without bioactive compounds. This is while most of the strains lost their resistance under the influence of the sub-MIC values of menthol and thymol. Overall, it was found that the strains showed the highest sensitivity to the antibiotics Imipenem (IPM10) and Aztreonam (AZT30), while all of them were resistant to Ceftazidime (CAZ30) in the susceptibility test with both compounds.

### CONCLUSION

The present study shows that menthol and thymol can be introduced as promising inhibitors against different strains of *Pseudomonas aeruginosa*.

**Keywords:** Antibiogram, Antibiotic resistance, Bioactive compounds, *Pseudomonas aeruginosa*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Blowfly Larvae Recognize where they Are (Wound or Corpse)?

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

A number of dangerous bacteria such as E.coli , MRSA, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis and some other bacteria are active in the wound and due to the many reasons, in chronic wounds the human immune system is not responsive and infection in the wound cause limb amputation or death of the patient! But fortunately, the larvae of blowfly very quickly destroys the biofilm that exists in all chronic wounds, and cures osteomyelitis in a very short period of time. During maggot therapy it is observed that the ESR and CRP of the patient decreased every day! In chronic wounds, where pathogenic bacteria are very active, the microbial toxins cause great damage to the tissues in the wound, but as soon as the larvae are placed in the wound, caused destroying microbes and microbial toxins. It is necessary for the larvae to destroy microbes, microbial toxins, biofilm formed in the wound, dead and necrotic tissues, and secrete growth factors to heal the wound. Fortunately, one of the important character of the larvae is that they recognize where they are working! In corpse they have the maximum secretion of proteolytic enzyme, and the secretion of this enzyme in the wound is regulated. It is necessary that the larvae heal the wound, but do not cause any damage to the healthy parts of the wound. If the larvae have the maximum secretion of proteolytic enzyme in the wound, it will cause the wound to enlarge and larvae will never be able to heal the wound. Fortunately, when the larvae are active in the corpse, they have the maximum secretion of proteolytic enzyme, and when they are active in the wound, the secretion of the proteolytic enzyme is regulated.

### MATERIALS AND METHODS

In laboratory, as soon the technician provide a piece of meat to the larvae, meat turns into a soap very quickly. By secreting a proteolytic enzyme, the larvae turn dead and necrotic tissues into soap. Larvae are not able to chew food, and it is necessary to turn the dead and necrotic tissues into a soapy form and then sucking this soapy food.

### RESULTS AND DISCUSSION

Fortunately, as soon as granulation tissue appears in the wound, the larvae leave the wound, and when the larvae leave the wound, they die because food is not available to them. In other words, the larvae prefer death to damage the granulation tissue.

### CONCLUSION

Larvae not only recognize where there are(in wound or corpse)?But, also larvae easily recognize black, yellow and pink wounds, and the level of proteolytic enzyme secreted by the blowfly larvae is different in black, yellow and pink wounds. The black wound is the most dangerous wound, so the level of proteolytic enzyme secretion by the blowfly larvae is higher in black wound than in the yellow and pink wounds, also the level of proteolytic enzyme secretion is higher in the first days of inflammation phase of a chronic wound than in the end stage of inflammation phase. Fortunately, the blowfly larvae can regulate the level of proteolytic enzyme secretion, and when the larvae works on the dead body of any mammals, birds, reptiles and all other creatures, the secretion of proteolytic enzyme will be more than black wounds.

**Keywords: Maggot Debridement Therapy (MDT) - Wound Healing - Granulation Tissue...**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Genotypic Analysis of Uropathogenic *Escherichia coli* Isolated from Urinary Tract Infections

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Escherichia coli* is the most predominant causative agent of urinary tract infection (UTIs) and nosocomial infections worldwide. *E. coli* strains are classified into eight phylogenetic groups A, B1, B2, C, D, E, F, and Clade I based on pathogenic genes. The severity of UTIs caused by *E. coli* strains depends on virulence genes such as the iron acquisition system genes, which are expressed at a high rate during infection. This study was aimed to assess the frequency of iron acquisition genes in phylogenetic groups of *E. coli* strains isolated from urine samples of patients with UTIs.

### MATERIALS AND METHODS

From April to March 2019, 193 *E. coli* were isolated from urine samples patients were referred to Razi Hospital in Rasht and confirmed by conventional microbiological methods. Then the DNA of isolates was extracted by the Boiling method. Phylogenetic grouping of *E. coli* strains was performed using the quadruplex PCR method according to the presence and/or absence of *chuA*, *yjaA*, and *arpA* genes and TspE4.C2. Then, multiplex PCR was used for detection of iron acquisition (*cirA*, *fiu*, *fuyA*, *ireA*, *irp2*, *iutA*, *mntH*, *sitD*, *fur*, *hma*, *sitA*, *sitB*, *sitC*) genes.

### RESULTS AND DISCUSSION

Out of 193 strains of *E. coli* isolated from urine samples of patients with UTIs, 91 (47%) isolates were in phylogenetic group B2 and then 39 (20.2%) Unknown and 25(13%) B1, respectively. The three genes *cirA*, *hma* and *sitD* had the highest frequency in all phylogenetic groups. Also, *mntH* and *sitA* genes were observed in phylogenetic group B2 more than other groups.

### CONCLUSION

The results of present study showed that the phylogenetic group B2 had the highest frequency among the studied *E. coli* strains. Also, the two genes including *mntH*, and *sitA*, which are more abundant in the B2 phylogenetic group, can be examined at the expression level.

**Keywords:** *Escherichia coli*; Phylogenetic group; Urinary tract infections; Multiplex PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Survey the Prevalence and Antibiotic Susceptibility Pattern of Uropathogens Isolates from Outpatients in North of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Urinary tract infections are one of the most common bacterial infections and are a health problem worldwide. Bacterial agents that cause urinary tract infections include a wide range of gram-negative and gram-positive bacteria. Current treatments for urinary tract infections are less than desirable, and the prevalence of multidrug-resistant uropathogens is increasing.

The aim of this study was to evaluate the frequency of uropathogenic strains isolated from outpatients with urinary tract infections and also to evaluate the patterns of antibiotic resistance among them.

### MATERIALS AND METHODS

Uropathogenic isolates were collected from urine among outpatients who were admitted to Razi hospital, Rasht, Iran, between 2020 until 2021. Identification of uropathogenic strains was performed according to bacteriological and PCR criteria. The pattern of antibiotic resistance of each of the isolated uropathogenic isolates was evaluated using Kirby-Bauer method.

### RESULTS AND DISCUSSION

Out of 164 outpatients with urinary tract infections, the prevalence of infection among women was 76.2% and the majority of patients (54.3%) were in the age group of 60 to 80 years and most patients had a history of recurrence of infection (75%). *Escherichia coli* 136 (82.9%) followed by *Klebsiella pneumoniae* 16 (9.8%) *Staphylococcus aureus* 6 (3.7%), *Enterococcus* species 4 (2.4%) and *Enterobacter* species 2 (1.2%) were identified as the most uropathogenic isolates. The results revealed that the rate of ESBL-producing isolates was 58.4%. Among gram-negative uropathogenic strains and producing ESBLs strains, the highest resistance was to ampicillin with 88.5%, ciprofloxacin with 66.3% and Trimethoprim-sulfamethoxazole with 63.8% and the highest susceptibility to fosfomycin with 82.8%, meropenem with 83.1% and imipenem with 79.9%. Gram-positive cocci isolated from urinary tract infections were resistant to penicillin and sensitive to vancomycin.

### CONCLUSION

Our results showed a remarkable rate of drug resistance among uropathogenic isolates from outpatients with UTIs in our studied region. Our study suggests the necessity of monitoring an empirical use of antibiotics at a geographical area in order to select an appropriate empirical treatment.

**Keywords:** Urinary Tract Infection, Uropathogen, Antibiotic Resistance, Outpatient

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Safety assessment of Lactic acid bacteria isolated from Iranian traditional fermented camel milk (Chal)

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

FAO/WHO defines probiotics as live microorganisms, when administered in adequate amounts confer a health benefit on the host. There is a decade debate on the health benefits of probiotics but because of the history of use they are generally considered safe. However, there are concerns over infectious disease caused by lactobacilli in patients with underlying diseases and emergence of vancomycin resistant enterococci (VRE) which could cause infection in hospitalized patients. Recently, FAO/WHO has published a draft guideline for evaluations of probiotics which includes safety assessment and functional characterization. The working group suggested a few tests to consider the microorganisms as safe, the most important of which was determination of antibiotic resistance patterns. In this research, potential probiotic strains including lactobacilli, enterococci, leuconostoc and weissella isolated from Iranian traditional fermented camel milk (Chal) were assessed for the incidence of virulence determinants, antibiotic susceptibility, and virulence phenotypes.

### MATERIALS AND METHODS

Total DNA of the 11 LAB strains was used to detect the presence of virulence genes, including *gelE*, *efaAfm*, *efaAfs*, *ace*, *espfs*, *cylM*, *cylA* and *cylB* using specific primers and PCR method. Antibiotic susceptibility was determined by commercially antibiotic-containing disks. Gelatinase, decarboxylase and hemolytic activities were determined using the phenotypic methods.

### RESULTS AND DISCUSSION

*CylB*, *gelE* and *efaAfs* were the most frequent genes identified in the strains. No  $\beta$ -hemolytic activity was observed while tyrosine decarboxylase activity and gelatinase production were observed in enterococcus and leuconostoc strains. Five out of 11 strains were resistant to both kanamycin and vancomycin.

### CONCLUSION

The results of this study suggested that according to the potential risk factors, traditional fermented foods need safety assessments in order to investigate their bacterial strains for harboring the virulence genes and transmissible resistance against antibiotics.

**Keywords:** Fermented Milk, Lactic Acid Bacteria, Safety Assessment, Virulence Determinants

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Inhibition of biofilm formation by serum raised to the outer membrane protein A of *Acinetobacter baumannii*

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## ABSTRACT

### BACKGROUND AND AIM

Biofilm production is one of the various virulence factors of *Acinetobacter baumannii* that helps the organism resist stressful conditions and facilitates the development of intense infections and diverse resistance mechanisms. Biofilms allow *A. baumannii* to survive and spread in hospital environments by attaching to different surfaces, such as cerebrospinal fluid shunts, and vascular catheters. Outer membrane protein A (OmpA) is a prominent porin in the outer membrane of *A. baumannii*. This protein plays a crucial role in bacterial pathogenesis by contributing to biofilm formation, interaction with epithelial cells, induction of apoptosis, and inhibition of complement, and serves as a potential vaccine candidate against *A. baumannii* infections.

### MATERIALS AND METHODS

We expressed recombinant protein OmpA (rOmpA). Purification was achieved using imidazole gradient of Ni-NTA column chromatography and confirmed with SDS-PAGE. The protein concentration was determined via Bradford colorimetric method. The purified OmpA was injected to BALB/c mice, and serum antibody titer was assessed using indirect ELISA. *A. baumannii* strains were exposed to OmpA serum, resuspended, and incubated in 96-well plates. Crystal violet staining and ELISA reader absorbance measurements followed after washing and decolorization with acetic acid.

### RESULTS AND DISCUSSION

The protein expression and purification were confirmed by analyzing the SDS-PAGE gel, which showed the protein at the expected position (approximately 38 kDa) and ensured correct protein folding. The indirect ELISA results indicated an increased antibody titer in immunized mice compared to the control group. When the serum was challenged with the bacteria, it was observed that the serum reduced biofilm production in both the standard and clinical strains of *A. baumannii*.

### CONCLUSION

The recombinant OmpA protein induced a protective effect against *A. baumannii* in mice and prevented biofilm formation in strains of *A. baumannii*. OmpA is considered as a promising subunit vaccine candidate against *A. baumannii* infections.

**Keywords:** *Acinetobacter baumannii*, OmpA, Biofilm formation, Subunit vaccine

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### A case report of *Mycobacterium tuberculosis* brain abscess identified in Payvand Specialty Clinical Laboratory

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#### ABSTRACT

##### BACKGROUND AND ABJECTIVE

A brain abscess is a pus-filled swelling in the brain. Tuberculosis (TB) causes by *Mycobacterium tuberclosis* (Mt) an acid fast bacterium which usually attack to lungs, but can invade any part of the body such as the kidney, spine, and brain. Opium addiction can cause life-threatening health problems such as brain hypoxia, coma and death. Herein, a case of brain abscess caused by Mt who referred to Payvand Specialty Clinical Laboratory is reporting.

##### MATERIALS AND METHODS

A sample of punctual fluid of brain abscess of a 57 years old man with opium addiction from a private hospital of Tehran were received to Payvand Specialty Clinical Laboratory during April 2023. In his history recording signs such as: fever, weight loose, sweating and cough were registered. Based on physician request direct smear and culture was done for acid fast bacilli. Direct smear evaluation was done by Rodamin florescent staining. Also, sample inoculated in a Lowenstein-Jensen media and incubated at 37°C for a month. Every other week the tube checked for colony formation.

##### RESULTS AND DISCUSSION

No growth of acid fast bacilli was detected in the first and second weeks. From the third week, the colonies started to grow and after fourth week a heavy growth of colonies were detected. In direct smear existence of acid fast bacilli reported as 3+ (>50 AFB) per field on average by florescent staining method.

##### CONCLUSION

Based on the study of Shamaei et al (2009) from Masih Daneshvari hospital Tehran, Iran the addiction rate among TB positive patients was to: opium 20.3%, heroin and opium together 2.8% and all three opium, heroin and crack 1.4%. Similarly, in Mirahmadizadeh (2022) study, the mortality rate of COVID-19 among opium addicted patients was higher than normal population. Also in Salehi study 2023 from Imam Khomeini hospital Tehran, 22% of pulmonary tuberculosis and 30% of extra-pulmonary tuberculosis patients were registered as opium addicted. Based on above studies, opium is common drug addiction which reported in Tehran, Iran. So, it seems drug addiction is a risk factor which can causes life threatening condition such as brain abscess of Mt infection.

**Keywords:** *Mycobacterium tuberclosis*, opium, brain abscess

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of Lipid Production by The Yeast Strains Isolated From Beaches of Qeshm Island

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Microbial lipids are of special importance in the food and pharmaceutical industries. Because the composition of their fatty acids is comparable to vegetable oils, they can be substituted by vital dietary lipids. Oleaginous yeast can be used to produce large quantities of valuable products at a low cost and is unaffected by weather and seasonal conditions. In general, yeasts are considered better than bacteria, molds, and algae due to their comparatively fast growth rate (in single cells), capacity to synthesize lipids in individual cells, and ability to absorb carbon sources from diverse industry byproducts.

This study focused on isolating yeast strains from water, soil, and plant samples to investigate their lipid biosynthesis ability and find a proper extraction method.

### MATERIALS AND METHODS

samples were cultured on YPD agar containing chloramphenicol at 25 °C for at least 72 hours, and subsequently evaluated for colony formation at 24hr intervals. Yeasts were isolated and Sudan Black B staining of the cells was carried out to observe the intracellular lipid particles. The screened yeasts were incubated in media containing KH<sub>2</sub>PO<sub>4</sub>, MgSO<sub>4</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, yeast extract, and glucose at 25 °C for 48 hours. Different cell disruption techniques and two solvent extraction methods were examined. Eventually, Cell disruption of the biomass was carried out by appropriate methods and lipid extraction was done using the proper solvent.

### RESULTS AND DISCUSSION

Ten yeast strains were isolated and four of them were selected based on the presence of intracellular lipid particles. They were identified, registered, and received Access numbers in the Iranian Research Organization for Science and Technology microbiological collection. Based on the results, freeze-thawing (repeated four times) followed by acid treatment at 60 °C was selected for cell disruption, and hexane was selected for lipid extraction. maximum lipid production was observed in the identified strains *Cystobasidium benthicum* PTCC5338 and *Filobasidium magnum* PTCC5339. *Hortaea werneckii* PTCC5337 and *Debaryomyces nepalensis* PTCC5336 produced lipids less than 20% dry cell weight.

### CONCLUSION

The Qeshm Island ecosystem has appropriate potential for the presence of oleaginous yeast strains.

**Keywords:** Cell disruption, Extraction, Lipid, Yeast

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The impact of co-inoculation with rhizobacteria and organic wastes on enhancing the plant growth and nutrient uptake in *Zea mays* L.

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Zea mays* L. is the leading cereal and fodder crop that is used for the production and consumption of food items. Synthetic fertilizers are used in conventional agriculture systems to obtain a high maize crop yield. But the frequent application of synthetic fertilizers causes deleterious effects on humans as well as excessive utilization of these fertilizers reduces plant nutrient uptake and soil fertility. PGPR together with organic waste could increase nutrient absorption and plant growth parameters. In this research, two rhizobacterial strains.

### MATERIALS AND METHODS

*Bacillus cereus* and *Pseudomonas putida* were isolated, molecularly characterized, and amplified by using 16s RNA primer and several biochemical tests were performed. A seed germination bioassay was performed to study the impact of PGPR on maize seed growth. Similarly, a pot experiment was executed to investigate the impact of *B. cereus*, *P. putida*, consortia (individually (3mL) and together with organic manure 50% V/V of CD (Cow dung) and VC (Vermicompost) on enhancing the plant growth parameters and nutrient uptake enhancement.

### RESULTS AND DISCUSSION

RB-1 strain was tested positive for HCN production, Catalase test, Oxidase test, while on the other hand it was tested negative for fluorescent test, (KOH) solubility test, levan production Test, carbohydrate fermentation test, (H<sub>2</sub>S) production test as well as for oxidative fermentative test. Similarly, RB-2 strains tested positive for all the tests except carbohydrate fermentation. Both strains were tested positive for IAA production (17.05 - 20.90 mg mL<sup>-1</sup>), phosphate solubilization (75-81 mg mL<sup>-1</sup>) as well as for siderophore production (24.53-28.55 %). Both strains, RB-1 identified as *Bacillus cereus* (MG027633.1) while RB-2 identified as *Pseudomonas putida* (MF462903-1). both rhizobacterial strains and bacterial consortia (*Sphingobacterium pakistanensis* sp., *Pantoea* sp., *Cellulomonas pakistanensis*., *Citrobacter* sp., *Exiguobacterium* sp., *Raoultella* sp., *Acinetobacter* sp., *Enterobacter* sp., *Alcaligenes pakistanensis*) showed positive results compared to control. All treatments showed maximum growth rate, biomass production, chlorophyll a and b, and carotenoid content, growth parameters and nutrient uptake ability in maize. The results showed that RB-1, RB-2, and consortia with the combination of CD and VC significantly ( $P < 0.05$ ) increased NPK, Mg, and Fe in shoot and root respectively compared to individual treatments as well as positive and negative control treatments.

### CONCLUSION

New combination with different organic waste manure with PGPR could give tremendously positive results for sustainable agriculture.

**Keywords:** PGPR, molecular and biochemical characterization, phylogenetic tree, *Zea mays*, nutrient Uptake, NPK

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Animals' susceptibility to SARS-CoV-2 and their role in the virus transmission, a One Health approach

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was the cause of the Covid-19 pandemic, which faced the world with many challenges. there are many hypotheses about the origin of this virus and most of them imply the animals' role in the transmission of this virus to humans which shows that the virus is zoonotic. Also, following various epidemiological studies conducted in the world, and also the close relationship between humans and animals (especially companion animals), concerns regarding about influential role of animals in transmitting the virus to humans intensified.

### MATERIALS AND METHODS

The articles have been collected from the PubMed database and Google Scholar. the keywords Covid-19, Animals, Pet animals, One Health, and Zoonosis were used for the search.

### RESULTS AND DISCUSSION

Overall, it is difficult to determine animals' roles in emerging zoonosis disease because of the lack of evidence, but there have been many seroepidemiological studies related to SARS-CoV-2 in different animal species due to the importance of this disease. according to the available evidence, 24 different species, including cats (*Felis catus*), dogs, hamsters, minks, and ferrets, can be sensitive to the virus.

### CONCLUSION

Many reports are stating that the virus can transmit between animals. cats are one of the most sensitive animals to the virus that are identified. according to this, there are high risks of reverse zoonotic cycles in these animals. moreover, other animals such as hamsters, minks, and ferrets can play important roles in the transmission virus to people. as demonstrated by reports, investigating the role of animals in the Covid-19 epidemic and the level of sensitivity of different animal species is important. based on this, molecular and serological techniques can be very helpful for determining the roles.

**Keywords:** Covid-19, Animals, Pet animals, One Health, Zoonosis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Comparison of low and high molecular weight biosurfactants effects on the biodegradation efficiency of petroleum hydrocarbons

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Low molecular weight (LMW) surface-active agents reduce surface and interfacial tension, and high molecular weight (HMW) types effectively emulsify and mix hydrophobic compounds in the aqueous phase (Kumar et al., 2021). This study was aimed to compare the efficiency of bacteria producing LMW and HMW biosurfactants in degrading petroleum hydrocarbons.

### MATERIALS AND METHODS

For this purpose, 18 bacterial isolates (isolated from different oil-contaminated areas) ability to produce LMW and HMW biosurfactants were evaluated based on the oil spreading and emulsion index ( $E_{24}$ ) tests, respectively. The ability of isolates to degrade petroleum hydrocarbons was also determined in a crude oil enriched mineral salt medium (Aparna et al., 2012). Finally, the LMW and HMW surface-active biomolecules producing ability correlated with the hydrocarbons biodegradation efficiency.

### RESULTS AND DISCUSSION

The results showed that seven isolates had a high ability to produce LMW biosurfactants and 11 isolates showed higher ability to produce HMW biosurfactants. Among them, three isolates were able to simultaneously produce LMW and HMW biosurfactants. The highest rate of oil degradation (92.2%) was observed in *Bacillus* sp. isolate, that had also the highest HMW biosurfactant production rate ( $E_{24}=99\%$ ). The correlation test revealed a significant negative and positive correlation ( $r=-0.45^*$  and  $r=0.58^{**}$ ) between degradin efficiency and LMW and HMW biosurfactants producing, respectively. HMW surface-active agents emulsify stably the hydrophobic compounds in the aqueous phase, cause a better mixing of hydrocarbons in the liquid environment, lead to an increased bioavailability of hydrocarbons and their subsequent biodegradation (Sakhaei et al., 2022). While LMW biosurfactants can improve the hydrocarbons desorption from soil mineral or organic matrix by reducing surface and interfacial tension (Bagheri et al., 2022).

### CONCLUSION

The results showed that bacteria producing HMW biosurfactants have a better ability to degrade hydrocarbons and can be considered as an effective indicator in the design and development of biological products to clean up polluted water sources. However, investigation of enzymatic activity and other associated traits with hydrocarbon degradation is necessary for better understanding and accurate selection.

**Keywords:** *Bacillus*, Correlation,  $E_{24}$ , microbial degradation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Effect of surface-active biomolecules on the dissolution and degradation of crude oil hydrocarbons

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Biosurfactants with bipolar nature and having two hydrophilic and lipophilic heads increase the microbial degradation of oil hydrocarbons by improving the dissolution and emulsion of these compounds in the aqueous phase (Fenibo et al., 2019). The aim of this study was to investigate the effect of biosurfactant production on the rate of crude oil biodegradation.

### MATERIALS AND METHODS

To achieve this, two isolated oil-degrading bacteria from oil-contaminated soil included *Pseudomonas aeruginosa* and *Ochrobactrum* sp. were selected and the biosurfactant production of isolates were evaluated by oil-spreading and phenol-sulfuric acid methods. Also, the thin layer chromatography (TLC) method was performed to determine the type of the produced biosurfactants. The isolates degrading ability was evaluated in a crude oil-enriched mineral salt medium in triplicate (Zhang et al., 2012).

### RESULTS AND DISCUSSION

The results showed that the oil spreading and biosurfactant production values of *P. aeruginosa* and *Ochrobactrum* sp. were 30 and 1 mm, 2.08 and 0.02 g/l, respectively. The oil degradation values of *P. aeruginosa* and *Ochrobactrum* sp. were obtained 39.9 and 22%, respectively. Also, the produced surface-active molecules was characterized as a glycolipid biosurfactant (rhamnolipid) based on the TLC test. The results showed that the production of biosurfactant by *P. aeruginosa* isolate led to the improvement of microbial degradation of petroleum hydrocarbons in liquid environment, which is likely due to the increase in the dissolution of hydrophobic compounds in the aqueous phase and the better accessibility of microbes for internalization and use as a source of carbon and energy (Rehman et al., 2021; Das et al., 2020).

### CONCLUSION

Production of *biosurfactant* by oil-degrading bacteria is an effective feature for improving the biodegradation of hydrocarbon compounds, which should be considered in the development of microbial products to remediate oil-contaminated resources.

**Keywords:** Emulsion, Glycolipid, Oil Spreading, *Pseudomonas*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Antimicrobial Activity of Methanolic Gonad Extracts of Persian Gulf Sea Urchin on Gram-Positive Bacteria

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The increasing spread of microbial resistance to current antibiotics has made the treatment of once easily treated small infections difficult. Marine animals and plants, along with their biological compounds, present a vast and accessible source for finding antimicrobial agents. In recent years, there has been an increase in studying the antimicrobial effects of marine organisms, such as echinoderms. Hence, this study was conducted to investigate the potential antimicrobial activity of the gonad extracts of sea urchin (*Echinometra mathaei*) found in the valuable biological reserves of the Persian Gulf.

#### MATERIALS AND METHODS

The study involved collecting *E. mathaei* from the Bushehr City coast of the Persian Gulf. The aqueous and Methanolic extracts were prepared from their gonads, and their antimicrobial effect was evaluated using the disc diffusion method and MIC and MBC assays against several clinical bacteria and their standard strains.

#### RESULTS AND DISCUSSION

The methanolic extract of the gonad of *E. mathaei* showed significant antimicrobial activity against bacteria. This extract demonstrated growth halos between 7-11.5 mm in *Staphylococcus aureus* ATCC6538, *S. aureus* clinical (WI), *Clostridium perfringens* clinical (G7S1), *Bacillus cereus* ATCC11778, *Listeria monocytogenes* ATCC19115, *Streptococcus sanguinis* ATCC10556, *S. agalactiae* PTCC1768. However, the examined extracts concentration prevented determining the MIC and MBC numbers.

#### CONCLUSION

The study concludes that *E. mathaei* gonad extracts can be a potential source of antimicrobial compounds for gram-positive pathogenic and opportunistic bacteria.

**Keywords:** Persian Gulf Sea Urchin, *Echinometra mathaei*, Antimicrobial Activity, Gram-Positive bacteria

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigation the esterase, proteinase, and hemolysin activity in *Candida* species isolated from patients with COVID-19 associated oral candidiasis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Oral candidiasis is a common disease in hospitalized patients with COVID-19. The present study was conducted with the aim of determining the production of enzymes involved in pathogenesis in *Candida* species isolated from patients with COVID-19 associated oral candidiasis hospitalized in Razi hospital, Rasht compared with healthy subjects.

### MATERIALS AND METHODS

This case-control study was conducted on 43 *Candida* species isolated from the oral swab samples of patients with COVID-19 associated oral candidiasis and 43 *Candida* species isolated from the oral swab samples of healthy people (normal flora). Enzyme activity index for important pathogenic factors including proteinase, esterase and hemolysin activity was measured using relevant protocols. p-value <0.05 with a confidence factor of 95% was considered statistically significant.

### RESULTS AND DISCUSSION

The results showed that the activity of esterase, proteinase and hemolysin of *Candida* species was significantly higher in patients with COVID-19 associated oral candidiasis compared to healthy individuals, and the activity of esterase and hemolysin enzymes in *Candida albicans* was higher in the both studied groups compared to non-albicans species.

### CONCLUSION

The present study showed that the *Candida* species isolated from patients with COVID-19 associated oral candidiasis and healthy individuals have the potential to produce enzymes involved in pathogenesis, but the activity and pathogenicity of these enzymes in the species isolated from patients is much higher than that of healthy individuals. Therefore, it can be concluded that commensal strains are opportunistic and are able to cause disease in favorable conditions such as COVID-19.

**Keywords:** COVID-19 Associated Oral Candidiasis, *Candida Species*, Hemolysin, Esterase, Proteinase.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of Chlorogenic Acid and Carnosol for Anti-Efflux Pump and Anti-Biofilm Activities against Clinical Extensively Drug-Resistant (XDR) Strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Efflux pumps and biofilm play significant roles in bacterial antibiotic resistance. This study investigates the potential of chlorogenic acid (CGA) and carnosol (CL), as phenolic and diterpene compounds, respectively, for their inhibitory effects on efflux pumps.

### MATERIALS AND METHODS

The presence of efflux pumps in clinical XDR strains of *S. aureus* and *P. aeruginosa* was screened using CCCP. Flowcytometry was used to examine efflux pump activity at sub-MIC concentrations of 1/8, 1/4, and 1/2 MIC, in comparison to the control. The inhibition of biofilm formation was assessed using the microtiter plate method. Finally, the MTT assay was conducted.

### RESULTS AND DISCUSSION

Among the 12 XDR strains of *S. aureus* and *P. aeruginosa*, 80% (4 out of 5) of the *S. aureus* strains and 85.7% (6 out of 7) of the *P. aeruginosa* strains exhibited active efflux pumps associated with gentamicin resistance. The checkerboard assay results, in combination with gentamicin, demonstrated that CGA exhibited a reduction in the MICs for XDR *S. aureus* strains. Similarly, CL showed a synergistic effect and reduced the MICs for both XDR strains of *S. aureus* and *P. aeruginosa*. Based on flowcytometric analyses, in XDR *S. aureus*, CGA demonstrated 39%, 70%, and 19% inhibition, while CL exhibited 74%, 63%, and 24% suppression. In XDR *P. aeruginosa*, CL exhibited inhibition rates of 25%, 10%, and 15%. Successful inhibition of biofilm formation was observed. And finally, MTT assay confirmed minimal cytotoxicity.

### CONCLUSION

Given the significant reduction in efflux pump activity and biofilm formation observed with CGA and CL in this study, these compounds can be considered as potential inhibitors of efflux pumps and biofilm formation, offering potential strategies to overcome antimicrobial resistance.

**Keywords:** ESKAPE, Chlorogenic Acid, Carnosol, Antimicrobial Resistance, Nosocomial Infection

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### High expression of IFN $\alpha$ and MDA-5 following SARS-CoV-2 infection

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

In 2019, the outbreak of coronavirus disease (COVID-19) happened in Wuhan, and its spread was faster than expected. As result, it is essential to differentiate the symptoms of COVID-19 in different age groups. Coronavirus is significant because it can lead to respiratory illnesses like sinusitis, laryngitis, pharyngitis, nasopharyngitis, and laryngotracheitis. These conditions affect the respiratory system and can cause cough, sore throat, and difficulty breathing.

#### MATERIALS AND METHODS

This cross-sectional study enrolled to evaluate the presence of SARS-CoV-2 in patients who referred to Khanevadeh hospital. All samples were tested with Real-time PCR, and the expression of genes such as RIG-1, MDA-5, and IFN $\alpha/\beta$  was evaluated. Consequently, all data were analyzed by SPSS and GraphPad prism.

#### RESULTS AND DISCUSSION

Of 138 patients, 22 (15.93%) cases were positive for SARS-CoV-2. All patients were children under 5 years old. 8 of them were male and 14 of them were female. The most common symptoms among patients were fever, cough, and runny nose and after that. The expression of IFN $\alpha$  and MDA-5 was significantly higher in COVID-19 patients.

#### CONCLUSION

The higher expression of MDA-5 and IFN $\alpha$  was found in positive COVID-19 patients which may show its importance in intrinsic immunity within the cell following SARS-CoV-2 infection compared to RIG-1. Sharp increased IFN $\alpha$  expression is seen following infection but there is slight change in IFN $\beta$  expression which we can conclude that maybe virus can prevent IFN $\beta$  expression effectively but cannot do it to IFN $\alpha$ .

**Keywords:** Innate immunity, RIG1, MDA5, COVID-19, IFN $\alpha$ , IFN $\beta$

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Survey the Antimicrobial Effects of Persica and Chlorhexidine Mouth Rinses against Cariogenic *Streptococcus* Species

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Mouthrinses play an important role in oral hygiene and prevention of tooth decay by reducing the proliferation of oral cariogenic flora. The purpose of this study was to investigate the antibacterial and anti-biofilm activity of Persica and chlorhexidine 0.2% mouthrinses against *Streptococcus mutans* and *Streptococcus sanguinis*.

### MATERIALS AND METHODS

To evaluate the antibacterial effect of mouthrinses on two standard strains of *S. mutans* and *S. sanguinis*, were used well diffusion and MIC methods and to examine their anti-biofilm effects, the microtiter plate method was used. Results were evaluated by Excel software 2019.

### RESULTS AND DISCUSSION

In the well diffusion method chlorhexidine 0.2% had an inhibition zone at concentrations of 500 mg/ml to 125 mg/ml, respectively. The MIC of Persica was determined at concentration of 500 mg/ml and chlorhexidine 0.2% at concentration of 31.25 mg/ml. In microtiter plate method, anti-biofilm activity was observed for chlorhexidine 0.2% at concentrations of 125 mg/ml to 31.25 mg/ml. Persica did not have any anti-biofilm effect on the studied bacteria.

### CONCLUSION

Chlorhexidine 0.2% mouthrinse has a very high antimicrobial power than Persica, but our study suggests that Persica mouthrinse if they are used in pure concentration, they can be a suitable alternative to chlorhexidine.

**Keywords:** Mouthrinse, Persica, Chlorhexidine, *S. Mutans*, *S. Sanguinis*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Prevalence of *Clostridioides difficile* contamination in the healthcare environment and instruments: A systematic review and meta-analysis

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

Worldwide, *Clostridioides difficile* infection is becoming one of the most common healthcare-associated infections (1,2). Management and control of this infection in healthcare facilities are associated with screening for environmental and instrumental *C. difficile* contamination (3). This systematic review and meta-analysis aimed to assess the overall prevalence of *C. difficile* in hospital settings, medical devices, and instruments.

##### MATERIALS AND METHODS

Four main databases, PubMed, Web of Science, Google Scholar, and Scopus, were searched using the keywords *Clostridioides difficile*, *Clostridium difficile*, *C. difficile*, clostridia, *Clostridium* spp., hospital environments, antibiotic associate colitis, intensive care unit, and ward in combination as a search strategy. The PRISMA checklist was used for selecting eligible studies.

##### RESULTS AND DISCUSSION

A total of 11 eligible articles published between 2012 and 2021 were included. The overall pooled prevalence of *C. difficile* in hospital environments was 14.9%. The highest and lowest prevalence were reported for India (51.1%) and the USA (1.6%), respectively. The highest prevalence was reported for beds (46.3%). A significant heterogeneity was seen between *C. difficile* prevalence in hospital environments in different samples. The highest and lowest prevalence was reported for floor corners (63.2%) and privacy curtains (1.4%), respectively.

##### CONCLUSION

In conclusion, hospitals' medical devices and environmental surfaces are considered a crucial source of *Clostridioides difficile* infection. In this regard, we strongly recommend revising and improving the cleaning and disinfection methods in hospitals and quality control of cleaning adequacy.

**Keywords:** *Clostridioides difficile*, healthcare environment, prevalence, meta-analysis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of Antibiotic Susceptibility Pattern of uropathogenic *Escherichia coli* among Kidney Transplant Recipients in North of Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Urinary tract infection (UTI) is the most frequent infection complication after kidney transplantation. Morbidity and mortality in kidney transplant recipients (KTRs) that are caused by UTI represents a substantial burden on health care resources. We aimed to investigate antibiotic susceptibility pattern of uropathogenic *Escherichia coli* (UPEC) in KTRs in North of Iran.

### MATERIALS AND METHODS

A total of 13 *E. coli* strains isolates were collected from urine among KTRs who were admitted to Razi hospital, Rasht, Iran, in 2021. The isolates were confirmed through phenotypic and genotypic methods. The disk diffusion method was performed for the antibiotic susceptibility pattern of isolates.

### RESULTS AND DISCUSSION

The results revealed that the rate of ESBL-producing and multidrug resistant (MDR) isolates was 76.9 % and 100%, respectively. Further analysis of antibiotic susceptibility among strains showed that the highest resistance rates were to ampicillin (92.3%), ciprofloxacin, cefuroxime and Levofloxacin (84.5%) and ceftriaxone (76.9%). On the other hand, the highest susceptibility in addition to carbapenems was toward fosfomycin and nitrofurantoin (92.3%), amikacin and Gentamicin (84.5%), ceftazidime and Piperacillin-tazobactam (76.9%). The ESBLs-producing strains shown significantly resistance to Cefuroxime, Cefepime, Ceftriaxone and ampicillin and susceptibility to nitrofurantoin ( $P<0.05$ ).

### CONCLUSION

Our results showed a remarkable rate of drug resistance among UPEC strains isolated from KTRs in our studied region. Our study suggests the necessity of monitoring an empirical use of antibiotics at a geographical area in order to select an appropriate empirical treatment.

**Keywords:** *Escherichia coli*, Kidney transplant, Antibiotic resistance, Urinary Tract Infection

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Effect of gamma ray and chitosan coating on microbial quality of fresh ground beef during storage at 4°C

First Roya Rafiee<sup>1\*</sup>, Fereshte Saeedi<sup>1</sup>, Azam Akhavan<sup>1</sup>, Khadijeh Ghotbi-Kohan<sup>1</sup>, Ramsina Betesho<sup>1</sup>, Akbari Hasan<sup>1</sup>

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

The aims of our research were to evaluate the effects of low doses of gamma irradiation (3-7 kGy) and chitosan coating on microbial quality including total bacterial populations and pathogens in ground beef.

### MATERIALS AND METHODS

Fresh raw ground beef samples included non-irradiated, coated with 2% chitosan (Ch-coated), irradiated (Cobalt-60 gamma-ray source), and finally ch-coated and irradiated samples (Ch-3-7 kGy). All samples were analyzed periodically.

### RESULTS AND DISCUSSION

The initial TVC (Day 0) of controls (non-irradiated) was 5.9 log cfu/g which reduced to 4.2, 5.1 and 3 respectively at the dose of 3 kGy, ch-coated and Ch-3 kGy samples. After 9 days of storage at 4°C, TVC was 7.3, 6, 5 and 3.8 log cfu/g respectively. There was a significant difference between the bioburden of control and irradiated samples with doses of 3 kGy, 5 kGy and 7 kGy in Ch-coated and Ch- non coated samples. But there was no a significant difference between 5 kGy and Ch-3 kGy, and between 7 kGy and Ch-5 kGy samples. In control, ch-coated and 3 kGy samples, initial *S. aureus* counts were 3.1, 1.9 and 1.6 log cfu/g which increased to 4, 2.5 and 2.6 respectively. *S. aureus* was not detected in other samples. In control and ch-coated samples, initial *E. coli* counts were 2.4 and 1.5 cfu/g which increased to 11 and 1.9 respectively. *Salmonella* spp. were only found in non-irradiated and ch-coated samples. It has been suggested that the mechanism of antibacterial activity of chitosan in Gram negative bacteria is disruption of the LPS layer of the outer membrane, and its action against *S. aureus* is due to increasing in membrane permeability, which leads to the leakage of cell inclusions.

### CONCLUSION

The results showed gamma radiation and chitosan had a great effect on reducing the initial bioburden of samples below the standard limit up to 7 days. Moreover, chitosan decreased the required absorbed dose to reach specific bioburden level. Therefore, gamma irradiation can be a valuable tool to reduce the economic losses related to the microbial spoilage. Since gamma radiation also affects biochemical and appearance characteristics, the optimal dose should be selected based on the results of a comprehensive investigation of the effect of gamma radiation on meat.

**Keywords: Pathogens, Gamma ray, Chitosan, Ground beef**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Molecular Survey of *Brucella melitensis* Field Isolates using Sequence-Based PCR of OMP31

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Brucellosis is a disease that imposes huge costs on the economy and society. It is one of the common diseases between humans and livestock in the world. Sequencing-based molecular methods have been introduced as an effective and repetitive method for bacterial strain typing, which can provide a reliable typing approach for clinical laboratories. The aim of this study is to describe the reproducibility and performance of the Outer Membrane Protein 31 (OMP31) based PCR, as a molecular genotyping tool for *Brucella melitensis* typing.

#### MATERIALS AND METHODS

In this study, 146 samples were taken from human blood samples, bovine and camel lymph nodes, as well as sheep and goat aborted fetuses, including fetal kidney, abomasum, liver, lung, spleen, and heart for bacteriological investigation. The molecular detection of the *Omp31* and *IS711* genes was performed using the isolated *B. melitensis* (n=14). The sequencing of the *Omp31* gene of *B. melitensis* in the Iranian field isolates was also performed for the whole gene sequencing. The homology of all sequences was then checked with the reported National Center for Biotechnology Information sequences using a basic local alignment search tool for the nucleotide diversity evaluation.

#### RESULTS AND DISCUSSION

The results showed that *Brucella melitensis* isolates were recovered from 14 examined cases and confirmed by IS711-based PCR method with PCR product of 731 bp. The 14 *Omp31* gene sequences were clustered as a single branch group with bootstrap support of 63, and they were closely correlated to the *Brucella melitensis* reference isolates which was determined in NCBI database.

#### CONCLUSION

Phylogenetic analysis based on OMP31 in animal and human hosts showed genomic similarity in isolates of different origins. Sequencing of this gene can also be used as a tool for identification of *Brucella melitensis* species and genus.

**Keywords:** OMP31, *Brucella Melitensis*, Phylogenetic analysis, Sequence-based PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Global prevalence of *Clostridioides difficile* in 17,148 food samples from 2009 to 2019: a systematic review and meta-analysis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Clostridioides (Clostridium) difficile* is an important infectious pathogen, which causes mild-to-severe gastrointestinal infections by creating resistant spores and producing toxins (1,2). Spores contaminated foods might be one of the most significant transmission ways of *C. difficile*-associated infections (3). This systematic review and meta-analysis study was conducted to investigate the prevalence of *C. difficile* in food.

### MATERIALS AND METHODS

Articles that published the prevalence of *C. difficile* in food in PubMed, Web of Science, and Scopus databases were retrieved using selected keywords between January 2009 and December 2019. Finally, 17,148 food samples from 60 studies from 20 countries were evaluated.

### RESULTS AND DISCUSSION

The overall prevalence of *C. difficile* in various foods was 6.3%. The highest and lowest levels of *C. Difficile* contamination were detected to seafood (10.3%) and side dishes (0.8%), respectively. The prevalence of *C. difficile* was 4% in cooked food, 6.2% in cooked chicken and 10% in cooked seafood.

### CONCLUSION

There is still little known concerning the food-borne impact of *C. difficile*, but the reported contamination might pose a public health risk. Therefore, to improve the food safety and prevent contamination with *C. difficile* spores, it is necessary to observe hygienic issues during foods preparation, cooking and transfer.

**Keywords:** *Clostridioides (Clostridium) difficile*, Food, Prevalence, Public health

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Evaluation and optimization of the SARS-CoV2 genomic RNA extraction

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

The new coronavirus (SARS-CoV2) is the cause of Covid-19, and according to the World Health Organization (WHO), has been a global problem and until last year, as a new epidemic, had covered all the countries of the world. This virus has a positive RNA of 29.8 kb. The most important diagnostic method for the new coronavirus is based on genome-based detection or real-time RT-PCR. For this purpose, specific parts of RNA virus are identified. Therefore, the first step for identification is the viral genome (RNA) extraction. In these viruses, extracting the genome with appropriate quality will be necessary for success in molecular RT-PCR methods. Therefore, various and sometimes field and mobile methods are used for the genome extraction of pathogens. The aim of this work is a simple and portable RNA extraction method for detection of SARS-CoV2 in the field.

##### MATERIALS AND METHODS

Pharyngeal contaminated samples with SARS-CoV-2 were obtained from Chamran Hospital and were initially confirmed using a specific test (real-time PCR). Then the genome (RNA) was extracted using filtered syringe and ImmunoMagnetic Separation (IMS) methods from a certain concentration of the infected sample containing SARS-CoV-2. Finally, the performance and sensitivity of the mentioned method in genome extraction were investigated using agarose electrophoresis, real-time RT-PCR, and RT-LAMP.

##### RESULTS AND DISCUSSION

The data indicated that the efficiency of two manual methods (filtered syringe and IMS) in extracting SARS-CoV2 viral genome can compete with commercial kits based on columns and centrifuges.

##### CONCLUSION

In conclusion, the innovative methods proposed in this research can be used for field and mobile extraction of viral genomes in order to perform molecular detection tests.

**Keywords:** Genome extraction, New coronavirus, Filtered Syringe, Magnetic Nanoparticles, Detection.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Frequency and Molecular Evaluation of Metallobetalactamase-Producing *Escherichia coli* in Medical Centers in Isfahan, Iran

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

This study was conducted to investigate the frequency and molecular evaluation of *Escherichia coli* bacteria producing metallobetalactamase enzymes isolated from healthcare centers in Isfahan.

### MATERIALS AND METHODS

A total of 3,500 clinical samples including wound, urine, respiratory, cerebrospinal fluid, and blood samples were collected from different wards of three hospitals in Isfahan. After sample collection, *E. coli* strains were identified using phenotypic, biochemical, and molecular tests. The antibiotic susceptibility pattern, MIC, in carbapenem-resistant isolates was determined using the E-test method, and the frequency of MDR, XDR, and PDR strains was determined. The MHT phenotypic method carried out on MDR strains and the presence of metallobetalactamase genes *bla<sub>IMP</sub>*, *bla<sub>NDM</sub>*, *bla<sub>VIM</sub>*, *bla<sub>SPM</sub>*, and *bla<sub>SIM</sub>* were investigated in MDR strains using PCR.

### RESULTS AND DISCUSSION

In the present study, 215 *E. coli* isolates were identified. In total, 160 isolates were isolated from urine, 5 from respiratory samples, and 26 from blood, 2 from cerebrospinal fluid, 18 from wounds or abscesses, and 4 from other clinical samples. The distribution of bacteria varied significantly depending on the type of sample ( $P < 0.001$ ). The most effective antibiotics against *E. coli* were amikacin (95.8%), colistin (100%), and tigecycline (95.8%). Additionally, the highest resistance was observed against ampicillin (94.9%). Nine isolates showed resistance to carbapenem antibiotics including imipenem, meropenem, doripenem, and ertapenem, which was confirmed by MIC results. In the MHT test, four *E. coli* isolates (1.8%) were positive. Of the total 215 *E. coli* isolates, 59.5% were MDR and 1.8% were XDR. Three *E. coli* isolates carried one of the genes *bla<sub>NDM-1</sub>*, *bla<sub>IMP-1</sub>*, and *bla<sub>VIM-1</sub>*.

### CONCLUSION

The findings of this study suggest that implementing systematic surveillance to identify *E. coli* producing metallobetalactamase and utilizing a rational approach to prescribing and using carbapenems may aid in the prevention of the prevalence of carbapenem resistance.

**Keywords:** Metallobetalactamase, *Escherichia coli*, Carbapenems

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation of *Sphingomonas paucimobilis* from an ocular infection and identification using ribosomal RNA gene: First case report from Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

This study reports a case of ocular infection caused by *Sphingomonas paucimobilis*, diagnosis, and its treatment using different antibiotics.

### CASE REPORT:

A middle-aged woman with prolonged purulent eye discharge was admitted to an ophthalmology clinic. A strain of *S. paucimobilis* was isolated from a patient who had been admitted to an ophthalmology clinic in Qazvin, Iran. The sample was identified by Sanger sequencing for the 16srRNA gene, and an antibiogram test was performed to determine its resistance profile. The patient was treated with ceftazidime and levofloxacin eye drops. The bacterial culture was negative 18 days after starting treatment with ceftazidime and levofloxacin. Antibiogram results showed that the isolated bacterium was resistant to aminoglycosides and colistin.

### CONCLUSION

The study highlights that *S. paucimobilis* can cause diseases even in immunocompetent individuals. Due to the different resistance profiles that this bacterium may have, treatment should be based on antibiogram results.

**Keywords:** *Sphingomonas Paucimobilis*, Ocular Infection, 16srrna Sequencing, Antibiotic Resistance, Case Report

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Assessing the Structure and Antimicrobial, Cytotoxic, and Apoptotic Properties of mCM11 Peptide Through Bioinformatics and Laboratory Experiments

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The objective of this study was to investigate the potential of antimicrobial peptides as a novel approach to combat drug-resistant infectious diseases.

### MATERIALS AND METHODS

The mCM11 peptide was designed by substituting lysine with arginine and amidating the C-terminal. The peptide was synthesized using the solid-phase method and evaluated by MS, HPLC, CD. The antimicrobial, cytotoxic, and apoptotic effects of the mCM11 were also investigated.

### RESULTS AND DISCUSSION

The mCM11 peptide exhibited a beta-sheet structure with a molecular weight of 1527.50 D and 96% purity. It displayed potent antimicrobial activity against both gram-negative and gram-positive bacteria with minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) ranging from 16 to 64 µg/ml and 16 to 128 µg/ml, respectively. The IC<sub>50</sub> of mCM11 was found to be 16 µg/ml, and its cytotoxicity in the SH-SY5Y cell line showed a dose-dependent manner. Moreover, apoptosis analysis of eukaryotic cells showed a decrease in late apoptosis and necrosis compared to untreated cells.

### CONCLUSION

The mCM11 peptide demonstrated significant antibacterial activity against a broad range of pathogenic bacterial strains without exhibiting any late apoptotic or necrotic effects on the eukaryotic cell line. These results suggest the potential of mCM11 as an effective therapeutic agent against drug-resistant infectious diseases.

**Keywords:** AMP, mCM11, Peptide, Bioinformatics, MBC, MIC

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Molecular identification of virulence gene *agfA* in different serovars of *Salmonella* isolated from human in Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Salmonella* is zoonotic bacteria and one of the most frequently isolated foodborne pathogens. Recently, there is no cost effective vaccine available, so it is a major worldwide public health concern. The *agfA* gene encodes AgfA protein that mediates the binding of *salmonella* to fibronectin, which was predicted to be 86% similar in primary sequence to the Escherichia coli curli structural protein (CsgA). The aim of this study was to analysis of the presence of *agfA* virulence gene in different serovars of *Salmonella* isolated from human in Iran, so that it can be used for diagnosis.

#### MATERIALS AND METHODS

A total 48 isolate of 11 different *Salmonella* serovars from the microbial collection of the Razi Vaccine and Serum Research Institute were used. Biochemical tests and serotyping were used to confirm the serovars. DNA extraction was performed by boiling method. Detection *agfA* gene (261bp) in different *Salmonella* serovars was performed by PCR then amplified products were analyzed by agarose gel electrophoresis.

#### RESULTS AND DISCUSSION

A total 36 out of 48 *Salmonella* isolates were positive (75%) for *agfA* gene. The results showed the presence of *agfA* gene was the most common in *S.paratyphiA* (90%), while low prevalence of this gene was observed in *S.paratyphiB* (42.8%).

#### CONCLUSION

The results indicated the presence of the *agfA* gene in different *Salmonella* serovars. The detection of this gene helps to understand the pathogenesis of *salmonella*. Moreover, presence of *agfA* gene in *S.paratyphiA* shows that they are specific target for molecular identification.

**Keywords:** *Salmonella* serovar-*agfA* gene-Virulence genes-PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The comparative study of microbial colonization of peri implant sulcus of implants restored with screw-retained superstructures: A cross sectional study

Mojtaba Bayani

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The aim of this study was to compare the microbial colonization of 10 types of microorganisms in the mucosal sulcus around healthy and infected implants.

### MATERIALS AND METHODS

In this cross-sectional study, 8 patients with two to three implants restored with screwed restorations and 3 years of follow-up, and with inclusion criteria were selected. Each of these patients had healthy and infected implants. Samples were taken under controlled conditions from the mucosal sulcus around the healthy and infected implants and the gingival sulcus of the adjacent tooth. Then it was sent to the laboratory to determine the presence of 10 microorganisms and underwent Polymerase Chain Reaction (PCR). The results were evaluated by stata 11 software.

### RESULTS AND DISCUSSION

The highest frequency of bacteria in the adjacent healthy tooth group belonged to *Campylobacter rectus*, *Prevotella intermedia*, *Fusobacterium nucleatum* and *Eikenella corrodens* and in the group of healthy screwed implants belonged to *Prevotella intermedia* bacteria, *Eikenella corrodens* and *Fusobacterium nucleatum* and in the group of infected implants belonged to *Prevotella intermedia*, *Eikenella corrodens*, *Fusobacterium nucleatum* *Treponema denticola* and the lowest frequency of bacteria in all 3 regions belonged to *Capnocytophaga*. It was not possible to calculate the significance level for any of the bacteria except in *Tanerella forsythia* ( $P < 0.05$ ).

### CONCLUSION

According to the results of this study, the total frequency of bacteria in infected screwed implants is higher than healthy screw implants. Also, there was not much difference between the presence of bacteria in the healthy tooth adjacent to the screwed and the healthy screwed implant.

**Keywords:** Colonization, Screwed prosthesis, Dental implant, Microbiology

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Bioremediation of phenol, sulfate sodium, and PAH by *Rhodococcus* sp. first time isolated and molecular characterized from aquatic and terrestrial ecosystems

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Environmental pollutions are the most significant problem worldwide. *Rhodococcus* sp. has a high potential for the production of secondary metabolites and degradation activity. This study aims to screen and characterize biodegradable *Rhodococcus* from Iranian ecosystems.

### MATERIALS AND METHODS

The *Rhodococcus* isolates were recovered from 90 environmental samples and identified using conventional and molecular methods. The growth rate in the presence of pollutants and chromatography (HPLC) was used to determine their biodegradation capability.

### RESULTS AND DISCUSSION

A total of 13 *Rhodococcus* isolates were characterized from the cultured samples (14.5%) that belonged to seven species. The prevalent species were: *R.erythropolis* (4 isolates;30.8%), *R.atherivorans* (3 isolates;23%), *R.ruber* (2 isolates;15.4), and *R.zopfi*, *R.phenolicus*, *R.equi*, and *R.rhodochrous* 1 isolate each. The result showed that these isolates could degrade and consume phenol, PAH, and sulfate sodium.

### CONCLUSION

Our results showed that the *Rhodococcus* species have significant potential for bioremediation of diverse types of pollutants. Therefore, more studies are recommended for the biodegradation activity of *Rhodococcus*.

**Keywords:** 16SrRNA gene sequencing; Biodegradation; *Rhodococcus*; HPLC

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### **Biofilm formation and cell adhesion of *Acinetobacter baumannii* ATCC 19606 and its clinical isolation on HeLa cervical epithelial cells in the presence and absence of anti-Oma87 antibody and acute cytotoxicity of antigen**

Zahra Panji and Iraj Rasooli

Shahed University, Tehran Iran

#### **ABSTRACT**

#### **BACKGROUND AND OBJECTIVES**

Bacterial resistance, especially resistance to *Acinetobacter baumannii*, has become one of the main problems of the medical community in the world today. Bacteria of the genus *Acinetobacter* are gram negative, completely aerobic, catalase positive and oxidase negative and due to continuous mutations and many resistances, they are considered one of the most important causes of human infections, especially in hospital environments.

#### **MATERIALS AND METHODS**

After expressing and purifying the outer membrane protein of *Acinetobacter baumannii* called oma 87 and injecting it into mice at different time intervals, blood was drawn from the mice in order to prepare anti-oma87 antibody and with the resulting serum, which contains a significant amount of anti-antibody oma87, biofilm, toxicity, attachment and internalization tests were performed. For example, the biofilm test was conducted in such a way that the amount of biofilm formation by two strains of *Acinetobacter baumannii*, one of which was a standard strain and the other a clinical strain, was checked in the presence and absence of mouse serum containing anti-oma87 antibody. Therefore, in the same way, the effect of serum containing antibody on the toxicity of *Acinetobacter baumannii* cells, the degree of adhesion of bacteria to the cell, and the entry of bacteria into the host cell were also performed on the mentioned strains, just like the biofilm test.

#### **RESULTS AND DISCUSSION**

After conducting the tests, it was found that the anti-Oma87 antibody is able to prevent the pathogenesis of *A. baumannii* bacteria.

**Keywords:** *Acinetobacter baumannii* , Oma87 , Biofilm , Attachment

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Anti-bacterial nano packaging and its effects on dairy products along with meat

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Meat and other dairy products are proper places for bacteria to increase, just as pathogens shows in meat, contains 90 percent of this group of diseases containing toxins and bacteria (Berhe G., Wasihun 2020). Diarrhea, bovine brucellosis are major foodborne diseases. World Health Organization (WHO) show that diarrheal diseases cause over 550 million infection and over 230,000 deaths a year (WHO, 2015). Therefore, we have many preventive methods to protect products from contamination, including freezing, pasteurization and packaging. Today, the development of food packaging is so important in preventing foodborne disease, and in the future can prevent worldwide pandemics and lead to have well-being product such as meat which can reduce the risk of poisoning by some bacteria.

### Advantage and disadvantage of old method packaging

In old method (Traditional packaging) many disadvantages like non-active barriers which can only defer contamination during packing (Brody et al., 2008), lack of strength as well as weakness against high and low temperature shown but it has positive side like chip cost, widely access to material, lead industry to use this method. Weakness of old packaging led studies to have a new kind of protection, nano packaging which have a great management on growing pathogens.

### Types of Anti-bacterial Nano packaging

We can separate Nano packaging into three major class; (i) Improved packaging: These packages contain NPs and can preventive to anti-bacterial, mechanical strength and also reduce needs of preservatives and other additives; (ii) Active packaging: this method containing preservatives like inorganic NPs and increase shelf life of food products and have active specifications such as anti-bacterial and anti-oxidants; (iii) Intelligent/smart packaging: this method of Packaging can manage biochemical or microbial changes and measures pathogen developing (Anvar, Ahari and Ataee, 2021) and can be so effective in way of long transportation.

### Advantage and disadvantage of nano packaging

Nanotechnology has several benefits for meat packaging beyond increasing shelf life, improve the barrier, mechanical, and heat-resistant properties of packaging, as well as its biodegradability, Nanoscale ingredients can added to meat products to improve taste and texture while masking off flavors, improve chemical stability of packaging, resistance to gasses, and water (Ramachandraiah K, Han SG, Chin KB, 2015).

## CONCLUSION

Nano packaging is a little expensive, but in the long term of use, observing the well-being, its more beneficial than others plans by necessity of increasing the shelf life of animal products in the food industry with a major role in the health of people worldwide. Studies shows success in developing this method and preserving products from foodborne pathogens and it will promote by decreasing costs with using economical material therefore mainly useful in transportation and storage of dairy products.

**Keywords:** Anti-bacterial, Nano packaging, Dairy product, Meat, Effects

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigation of the antibacterial properties of oregano against standard and resistant hospital isolates of *Staphylococcus aureus* and *Klebsiella pneumoniae*

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Bacterial infections and their transmission are a major threat to human health. Currently, antibiotics are the main treatment for these infections, which has inevitably led to the rapid emergence of antibiotic-resistant bacterial species in recent decades. It is therefore necessary to develop a new strategy to inactivate bacteria. Plant extracts containing antimicrobial compounds are good candidates for eliminating resistant bacteria. The aim of this study is to investigate the antimicrobial effects of oregano (*Origanum vulgare*) extract against *Staphylococcus aureus* and hospital-resistant *Klebsiella pneumoniae* bacteria.

#### MATERIALS AND METHODS

The alcoholic extract of the oregano plant was followed by MIC and MBC tests against two MDR and hospital standard isolates of *Klebsiella pneumoniae* and *Staphylococcus aureus*.

#### RESULTS AND DISCUSSION

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for standard bacteria is 0.5 mg/ml and also for resistant bacteria, *Staphylococcus aureus* is 1 mg/ml and for *Klebsiella pneumoniae* is 0.5 mg/ml.

#### CONCLUSION

Plant extracts with antimicrobial compounds can be used to destroy bacteria that are resistant to all types of antibiotics, significantly reducing the prevalence of antibiotic resistance.

**Keywords:** Antibacterial, Oregano, *Staphylococcus Aureus*, *Klebsiella Pneumoniae*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of dietary exposure to tetracycline, oxytetracycline, and chlortetracycline antibiotic residues in domestic animal's milk using the four-plate test, ELISA, and HPLC methods in Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

the health of unprocessed raw milk is especially important since it serves as the foundation for other dairy products. The use of antibiotics for optimal growth, development, and management of local infections in animals has become frequent. This study was conducted to determine the amount of tetracycline, oxytetracycline, and chlortetracycline in raw milk using FPT, ELISA and HPLC microbial methods.

### MATERIALS AND METHODS

This descriptive and analytical study was conducted cross-sectionally over two years with the cooperation of Veterinary Organization of Hamedan Province, Iran. 246 unprocessed raw milk samples were taken from milk collection centers, industrial and traditional cattle farms and transferred to the laboratory. In the laboratory, FPT was first performed to check the presence of antibiotics. Then, the positive samples were analyzed in terms of antibiotic residue by ELISA method and using its specific kit. In the last step, the HPLC method was used to accurately determine the type and amount of antibiotic residue.

### RESULTS AND DISCUSSION

FPT test results showed that out of 246 milk samples, 47 samples (19.11%) were positive for antimicrobial residues. The highest and lowest antibiotic residues detected were related to penicillins, tetracyclines, macrolides, and amino glucosides, respectively. The results of ELISA analysis showed that the amount of antibiotic residue in 14 samples (29.79%) was higher than MRL with an average of  $98.43 \pm 6.86$   $\mu\text{g}/\text{kg}$ . The residues of tetracyclines were also detectable in 29.78% of the positive samples by ELISA in HPLC analysis. The average amount of tetracyclines in these samples was  $105.73 \pm 7.25$   $\mu\text{g}/\text{kg}$ .

### CONCLUSION

The presence of antibiotic residues in animal products indicates the importance of monitoring the amount of antibiotic residues in milk and other food products of animal origin. It seems that the regulation and formulation of relevant national standards and regulations is necessary.

**Keywords:** Milk, Antibiotic residue, FPT, ELISA, HPLC, Tetracyclines

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# High prevalence of $\beta$ -lactam and aminoglycoside resistance in *Clostridium perfringens* isolates from food producing-animals in Kerman, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Clostridium perfringens* (*C. perfringens*) is a ubiquitous, anaerobic, Gram-positive and spore-forming bacteria, ranking amongst the most important pathogens living in the intestinal tracts of both humans and animals. Under certain conditions, *C. perfringens* may become an opportunistic pathogen and over-proliferate, producing a wide spectrum of toxins that can lead to its pathogenic effects. Meanwhile, *C. perfringens* is recognized as one of the most important foodborne pathogens in humans; and considered as one of the leading cause of nosocomial diarrhea. So, emergence of antibiotic-resistant strains makes the dealing with the bacteria more challenging. The objective of this study was to determine the resistance of *C. perfringens* isolates to  $\beta$ -lactam and aminoglycoside antibiotic classes.

### MATERIALS AND METHODS

A total of 273 *C. perfringens* isolates, which were previously recovered from fecal samples of the diseased goats and sheep from Kerman province in southeast of Iran, evaluated for antimicrobial resistancy against the penicillin ( $\beta$ -lactam) and streptomycin (aminoglycoside) antibiotics by Kirby-Bauer disk diffusion method.

### RESULTS AND DISCUSSION

The findings of our study demonstrated the high antibiotic resistant rate of *C. perfringens* isolates in food producing-animals. The highest resistancy were observed to streptomycin (83.51%) and then penicillin (41.02%) antibiotics, respectively.

### CONCLUSION

To the best of our knowledge, this is the first study regarding the antimicrobial susceptibility of *C. perfringens* in food-animals in Kerman province of Iran. The present findings provide supplementary substantiation for the potential involvement of animals as a reservoir for the resistant *C. perfringens* strains.

**Keywords:** *Clostridium perfringens*;  $\beta$ -lactam; Aminoglycoside; Penicillin; Streptomycin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Comparative Assessment of ELISA and MLD for Measurement of Epsilon Toxin in Enterotoxemia Vaccine Production Process

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#### ABSTRACT

One of the most common veterinary biologicals is enterotoxemia vaccines that produced against *Clostridium perfringens*. Enterotoxemia diseases are mediated by toxins and epsilon is the most pathogenic toxin so killed vaccines comprise toxoided forms of these toxins. During vaccine manufacture toxin production is measured by Minimum Lethal Dose (MLD) tests that rely on animal testing in mice that measures the minimum concentration of toxins.

The objective of the present study was to compare the performance of ELISA assay that quantify epsilon toxins and toxoids with MLD and correlation evaluation. Suitable *in vitro* assays would completely replace the use of animals in testing at these stages, furthermore ELISA test has the ability to detect toxin and toxoids while MLD detect the active toxins only.

20 supernatant sample of type D fermenter culture which contain active toxin used in this research for MLD test after activation with trypsin serial dilutions was done and for each dilution, 0.5 ml was inoculated intravenously into each of two mice. For ELISA assay microtitration plates coated with proper dilutions of supernatant samples and standard toxins as positive control and other stages was performed and results interpreted. Pearson correlation coefficient and linear regression analysis used for the correlation between MLD and ELISA results.

Correlation coefficient greater than zero indicates a positive relationship between two tests so precise replacements of ELISA by mouse tests in this stage would significantly reduce animal usage in vaccine production and is a suitable replacement for very painful, stressful and prone to high variability assay.

**Keywords:** *Clostridium perfringens*, Epsilon toxin, MLD, ELISA

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The in vitro anti-Leishmania effect of medicinal plants on the promastigote of *Leishmania major*

Seyedeh Mehrnaz Mostatabzadeh, Hossein Rezvan\*, Sahar Hamoon Navard, Hamidreza Heydarianpour, Mohadeseh Asgari

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Leishmania are protozoan parasites that affect approximately 2 million people worldwide. Current treatment for cutaneous leishmaniasis is not completely satisfactory and involves the use of pentavalent antimonials. However, chemical drugs have limitations due to disease relapse, drug resistance and severe side effects. Recently, the use of alternative treatments from medicinal plants has increased.

### MATERIALS AND METHODS

In this study, scientific databases such as PubMed, Science Direct, Google Scholar and domestic scientific databases in Iran have been studied from 2009 to 2021.

### RESULTS AND DISCUSSION

In studies conducted, medicinal plants such as *Cymbopogon* species, *Lippia sidoides* Cham, *Allium cepa*, *Zingiber officinale*, *Osmium gratissimum* and *Artemisia* species have shown activity in inhibiting the growth of promastigotes. These herbs were not significantly effective, when used alone. Changing the extraction methods or combining them with other drugs, may improve their efficacy. Some of these plants can also be used as a complementary treatment.

### CONCLUSION

Medicinal plants can have a significant impact on the treatment and reduction of side effects from cutaneous leishmaniasis. These herbs can be used as alternative treatment to chemical drugs. However, further studies are needed to in-vivo evaluation.

**Keywords:** Cutaneous leishmaniasis, Herbal medicine, Promastigote

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Identification and molecular investigation of *Campylobacter jejuni* isolated from poultry and human samples in Gilan province

Fatemeh (Elham) Alikhani<sup>1</sup>, Mohammad Faezi Ghasemi<sup>1\*</sup>, Nour Amirzafari<sup>2</sup>, Abbas Yadegar<sup>3</sup>, Arash Chaichi Nosrati<sup>1</sup>

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Campylobacter jejuni* infection is one of the most common and one of the main causes of bacterial foodborne diseases worldwide and can lead to gastroenteritis in humans and its symptoms range from mild diarrhea cramps and severe abdominal fever. It is usually found in poultry and is often transmitted to humans through the consumption of undercooked chicken. In recent years, there has been increasing concern about the prevalence and pathogenicity *C. jejuni* strains isolated from chicken meat samples, raising questions about a possible link between chicken meat consumption and human infections. Therefore, in this study, the prevalence, virulence gene profile, and molecular and phylogenetic characteristics of *C. jejuni* isolated from chickens and humans from different cities of Gilan were evaluated.

### MATERIALS AND METHODS

*Campylobacter* strains were isolated during a 1-year sampling (August 2021 to August 2022). Poultry Cloaca samples were collected from 8 slaughterhouses and local retailers. Human samples were isolated from 8 centers in the clinical region. All *C. jejuni* strains were identified by standard microbiological methods and confirmed by targeting *cadF*, *flaA* and *porA* genes using PCR.

### RESULTS AND DISCUSSION

Out of 173 *Campylobacter* isolates, 152 strains were isolated from poultry and 21 cases were isolated from sporadic cases of children's diarrhea. Feces and chicken meat had the highest prevalence of *Campylobacter* (65% and 55.83%). *Campylobacter* was observed in 17.5% of environmental samples of slaughterhouses and poultry farms and 23.07% of human samples. Based on PCR data, *C. jejuni* isolates had 97% *cadF*, 93.8% *flaA* and 91.3% *ciaB* virulence genes. Also, phylogenetic analysis showed that some *C. jejuni* isolates from chicken and human samples were similar.

### CONCLUSION

The present results support the possible risk of transmission of highly dangerous *C. jejuni* as a foodborne pathogen from chicken meat to humans. Active biosecurity measures on chicken farms and more sanitary efforts in industry and traditional chicken slaughterhouses should be implemented to effectively control this foodborne disease.

**Keywords:** *Campylobacter jejuni*, Gilan province, Phylogenetic relationships

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Identification of some genes associated with virulence and antibiotic resistance in *Campylobacter jejuni* by using bioinformatics tools

**Mahlagha cheraghi<sup>1</sup>, Hossein Sahragard<sup>1</sup>, Nastaran Asghari Moghaddam<sup>1\*</sup>, Bita Bakhshi<sup>2</sup>**

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Campylobacter jejuni*, a zoonotic pathogen, is widely acknowledged as a significant contributor to global foodborne bacterial illnesses. In addition, there is growing evidence that Guillain-Barré syndrome, an inflammatory demyelinating disease of the peripheral nervous system, is frequently preceded by *C.jejuni* infection. Virulence and antibiotic resistance can manifest in various adaptive, innate, and acquired forms. The intricate regulation of genes responsible for both antibiotic resistance and virulence is interconnected and influenced by environmental factors, posing challenges for gene regulation. Key mechanisms involved in regulation establish a connection between antibiotic resistance and virulence. This study aimed to investigate the expression of virulence genes and antibiotic resistance determinants in *C.jejuni*, by using bioinformatics analysis as well as to investigate the relationship between these two traits.

#### MATERIALS AND METHODS

The microarray data with the accession IDs GSE28295 and GSE17881 were identified, extracted and analyzed by GEO2R online tools and R software. Genes with the highest differential expression were identified using parameters  $P < 0.05$  and  $LogFC > |1|$ . Subsequently, the expression of the related genes was isolated and for the genes that had an increase in expression, the protein network was predicted by STRING database and visualized with the Cytoscape software.

#### RESULTS AND DISCUSSION

By identifying different genes that had a significant decrease or increase in expression, it was found that a set of genes (*acnB*, *gltA*, *sdhA*, *sdhB*) are involved in virulence, while another set of genes (*flgB*, *CmeC*, *sdhA*, *sdhB*) may contribute to the antibiotic resistance of *C.jejuni*. Additionally, a relationship between *sdhA* and *sdhB* genes in both virulence and antibiotic resistance to Erythromycin was revealed.

#### CONCLUSION

The findings of this study by utilizing bioinformatics tools, highlight the complexity of the relationship between virulence and antibiotic resistance in *C. jejuni*, which has become a major concern to the emergence of multidrug-resistant strains. Further investigation is needed to better understand *Campylobacter* pathogenesis, with an approach considering the interactions between virulence markers and antibacterial resistance.

**Keywords:** *Campylobacter jejuni*, antibiotic resistance, virulence, microarray, bioinformatics analysis.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Prevalence of Extended-Spectrum $\beta$ -Lactamase in *Pseudomonas aeruginosa* Isolated from distinct Clinical specimen

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Pseudomonas aeruginosa* is a one of the most accepted causes of nosocomial infections. Resistance of *P. aeruginosa* to  $\beta$ -lactam antibiotics may be the result of acquired resistance through mutation and over production of various antibiotic inactivating enzymes. This probe aimed to rule the prevalence of extended-spectrum  $\beta$ -lactamases (ESBL) production as well as the presence of their related genes *P. aeruginosa* isolated from patients,

### MATERIALS AND METHODS

The antimicrobial susceptibility of 20 *P. aeruginosa* isolates from patients was examined against 13 antibiotics by the disc diffusion method.  $\beta$ -lactam/inhibitor (BLI) combined disks including ceftazidime/clavulanic acid and cefepime/clavulanic acid were considered for isolates that revealed resistance phenotype to cefepime and ceftazidime to detect ESBLs genes, multiplex PCR was done.

### RESULTS AND DISCUSSION

Imipenem showed The highest resistance 100% ,while the highest sensitivity to the antibiotic was Gentamycin (55%) was observed.ESBL were observed in 8(%53). Overall, 15 isolates (75%) harbored bla AmpC (ICUs wards) , 1 (5%) had bla TEM and 1 (5%) carried bla PER genes. harbored bla AmpC , had bla PER 1 (5%) gene and bla SHV and bla OXA genes were not detected .

### CONCLUSION

A high prevalence of multiple  $\beta$ -lactamase production was observed among the AmpC producers (75%). that the highest prevalence was observed in the intensive care unit and this conclusion emphasizes the correct implementation and monitoring of relevant protocols.

**Keywords:** Beta-Lactamase; *Pseudomonas aeruginosa*; blaESBL

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Comparison of physicochemical properties of lipase in *Pseudomonas aeruginosa* isolated from wound and oil contaminated soil.

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

*Pseudomonas aeruginosa* is a Gram-negative and opportunistic bacterium. Large number of pathogenic factors and high antibiotic resistance make it very difficult to eradicate this bacterium from lung infections of cystic fibrosis patients. This bacterium is widely used in industry and has a number of active extracellular enzymes. In this research, a comparison has been made between *Pseudomonas* lipase isolated from a patient's wound and oil-contaminated soil.

#### MATERIALS AND METHODS

First, two strains were cultured in a salt-based medium with olive oil as a carbon source. After 72 hours, the medium was centrifuged and the supernatant was used as a source of lipase. The enzyme activity was compared at different pH, temperature and ionic strength.

#### RESULTS AND DISCUSSION

The results showed that in the wound strain, the highest and lowest activity was seen at pH 9 and 4, respectively, while in the soil strain, the highest and lowest activity was seen at pH 8 and 6. The study of the effect of temperature on lipase activity showed that in the wound strain, the highest and lowest activity was observed at 60 and 70°C, respectively, while in the soil strain, the highest activity was observed at 30°C and the lowest at 60°C. The effect of changing the ionic strength (different NaCl concentration) on lipase showed that in the wound strain, the highest and lowest activity was observed in 0.3 and 1 M NaCl, respectively, while in the soil strain, the highest activity was observed in 0.5 M and the lowest in 1 M NaCl.

#### CONCLUSION

The comparison of the above results shows that the physicochemical behaviours of the lipase enzyme from the wound and soil strains is completely different from each other, and it can be suggested that the structure of the secreted lipase from the wound strain is different from the structure of the enzyme from the soil strain.

**Keywords:** Lipase · wound · soil, bacteria

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Bioluminescence in Food Packaging: A Bright Future for Food Safety

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### ABSTRACT

Bioluminescence is a fascinating natural phenomenon that involves the conversion of chemical energy into light energy by an enzyme called luciferase. Many organisms in nature, such as bacteria, fireflies, and jellyfish, possess the ability to emit light through this mechanism. This unique property of bioluminescent organisms has been extensively studied and characterized, laying the foundation for its application in various fields, including food packaging. One of the most well-known applications of bioluminescence in food packaging is the ATP-assay, which utilizes the activity of firefly luciferase. Adenosine triphosphate (ATP), a molecule present in all living cells, serves as an indicator of microbial presence. The ATP levels are proportional to the number of cells present, making it a reliable marker for estimating microbial load in packaged foods. By measuring the light emitted through the luciferase/luciferin reaction following ATP extraction, the bioluminescent assay provides a rapid and accurate estimation of cell populations. ATP bioluminescence has been successfully applied in various food products, including milk, poultry, meat, and produce. The assay can provide results within approximately 15 minutes, offering a real-time assessment of microbial contamination. This rapid detection method has significant implications for ensuring the safety and quality of packaged foods, allowing for timely interventions if contamination thresholds are exceeded. Also, the use of this method, in addition to many advantages, also has disadvantages, and solutions have been provided to improve these defects, as well as to improve its performance and sensitivity.

### BACKGROUND AND OBJECTIVES

In recent years, bioluminescence has emerged as a revolutionary technique in the field of food packaging. By harnessing the natural phenomenon of light emission from living organisms, scientists have developed innovative methods to detect food spoilage and ensure the safety and quality of packaged products.

### MATERIALS AND METHODS

In this method, we extract luciferin and luciferase proteins from fireflies and bioluminescent microorganisms and add them to food packaging during a process.

### RESULTS AND DISCUSSION

In addition to being safe and cost-effective, this method has great advantages such as high accuracy and speed, so it is a great method for quick and accurate microbial measurement of food.

### CONCLUSION

As bioluminescence continues to captivate the scientific community, exciting opportunities emerge for its further application in food packaging. Ongoing research aims to enhance the sensitivity and specificity of bioluminescent assays, enabling more accurate detection of microbial contaminants. Additionally, the development of novel biosensors and the exploration of phage-based diagnostics open doors for targeted and efficient pathogen detection in food products. The versatility and potential of bioluminescence in the food packaging industry hold great promise for advancing food safety practices and ensuring the delivery of high-quality, uncontaminated products to consumers.

**Keywords:** Bioluminescence, ATP assay by bioluminescence, Bioluminescence in Food Packaging, food packaging

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Comparative Analysis of Squalene Production in *Cystobasidium benthicum* and *Saccharomyces cerevisiae*

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## ABSTRACT

### Background and Objectives

Squalene is a versatile linear polyunsaturated triterpenoid with diverse applications in the cosmetic and pharmaceutical industries. It can be used as a vaccine adjuvant component, a nutritional supplement, and an antioxidant. Yeast cells have a great potential to serve as a source for squalene production. This investigation aims to evaluate the content of squalene in two yeast strains: *Cystobasidium benthicum*, isolated from beaches of Qeshm Island, and *Saccharomyces cerevisiae*, in the presence of terbinafine as an inhibitor, and at different temperatures.

### Materials and Methods

Wild-Type *Saccharomyces cerevisiae* and native *Cystobasidium benthicum* cells were cultured in a medium containing glucose, yeast extract, and a nitrogen compound. Terbinafine, an inhibitor of ergosterol biosynthesis, was added to the culture medium at concentrations ranging from 0.02 to 0.55 mM. Biomass formation and squalene content in these yeasts were analyzed after 24, 48, 72, and 96 hours of fermentation. To analyze the effect of temperature on squalene production, these yeasts were cultivated at temperatures ranging from 10°C to 35°C. All experiments were performed in biological triplicates.

### Results and Discussion

In the absence of the inhibitor, *Saccharomyces cerevisiae* and *Cystobasidium benthicum* produced squalene at rates of 1.6 and 0.12 mg/g dry cell weight (DCW), respectively. Terbinafine affected the biosynthesis of squalene in both yeast strains. Maximum squalene content in *Saccharomyces cerevisiae* ( $10.02 \pm 0.53$  mg/g DCW) and squalene yield ( $20.70 \pm 1.00$  mg/L) were achieved using 0.442 mM terbinafine after 28 hours and 0.300 mM terbinafine after 30 hours, respectively. Maximum squalene content in *Cystobasidium benthicum* ( $11.32 \pm 1.97$  mg/g DCW) and squalene yield ( $12.01 \pm 1.11$  mg/L) were achieved after 96 hours using 0.258 mM and 0.277 mM terbinafine, respectively. The Temperature was found to be a significant factor affecting squalene synthesis in both strains. An increase in temperature led to a reduction of squalene production by *Saccharomyces cerevisiae* and an increase by *Cystobasidium benthicum*.

### Conclusion

The present study demonstrated that *Saccharomyces cerevisiae* and *Cystobasidium benthicum* are both potential sources of squalene production. *Saccharomyces cerevisiae* is a safe and versatile yeast that can adapt well to a controlled fermentation environment and grow quickly, making it a favorable option for commercial production. While *Cystobasidium benthicum* may not be as widely available, it can compete with *Saccharomyces cerevisiae* in terms of squalene production and is a suitable strain for this purpose.

**Keywords:** *Saccharomyces cerevisiae*, Squalene, Inhibitors, Temperature

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the effect of *Lactobacillus acidophilus* consumption on the biological pathways of the immune system

**Mehdi Abbasi<sup>1\*</sup>, Tannaz Alipour<sup>2</sup>, Nasrin Hajizadeh<sup>3</sup>**

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Probiotics are microbial strains that are beneficial for health. The word probiotic is derived from a Greek word that means "for life". Their first advantage is that the digestive system can control diarrhea, reduce lactose sensitivity, and minimize cholesterol absorption. Probiotics modulate the intestinal microbial composition and regulate the mucosal immune response through the induction of various cytokines. The aim of this study was to investigate the gene expression profile of *Lactobacillus acidophilus* consumers and its effect on the immune system.

### MATERIALS AND METHODS

In this part of the study, to investigate the expression of genes in the use of *Lactobacillus acidophilus* probiotic compared to the healthy intestinal mucosa, a microarray dataset from the GEO database was studied. After normalization, the desired data sets are analyzed by PCA and boxplot. Significant genes are obtained with the help of limma software package in R software. Genes with an absolute value (Log Fold Change, LFC) greater than 1 and FDR value  $\leq 0.05$  and t-test will be selected as significant genes.

### RESULTS AND DISCUSSION

GSEA analysis is done in order to determine biological pathways, determining biological pathways is usually done with the aim of enriching genes and displaying their functional information and revealing the biological mechanisms guided by these genes. For this purpose, TopGene database was used to determine the biological pathways of significant genes. Dataset No. GSE18741 was selected for this study, which contained 28 samples, in which a comparison was made between control samples or healthy individuals (8 samples) and samples or individuals consuming *Lactobacillus acidophilus* (8 samples), finally 4 significant genes were found (Table 1) that all 4 genes were highly expressed in people who consumed *Lactobacillus acidophilus*.

### CONCLUSION

One of the main mechanisms of action of probiotics is the regulation of the host's immune response. In contrast, the innate system responds to common structures, called pathogen-associated molecular patterns (PAMPs), shared by the majority of pathogens. In this study, the effect of consuming *Lactobacillus acidophilus* on the intestine and changing the gene expression profile was investigated. Probiotic bacteria are a special example of bacterial species that include a common part of the human microbiota.

GSEA analysis is done in order to determine biological pathways, determining biological pathways is usually done with the aim of enriching genes and displaying their functional information and revealing the biological mechanisms guided by these genes. Pathway analysis not only follows the biological pathways of a specific set of expressed genes, but also identifies the relationships between these genes.

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For this purpose, ToppGene database was used to determine the biological pathways of significant genes [10]. Dataset No. GSE18741 was selected for this study, which contained 28 samples, in which a comparison was made between control samples or healthy individuals (8 samples) and samples or individuals consuming *Lactobacillus acidophilus* (8 samples), finally 4 significant genes It was found (Table 1) that all 4 genes were highly expressed in people who consumed *Lactobacillus acidophilus*.

**Table 1: Significant genes obtained in the present study**

Gene Symbol	Description
ZC3H12A	Zinc finger CCCH-type containing 12A
CCL20	Chemokine (C-C motif) ligand 20
CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1
CXCL2	Chemokine (C-X-C motif) ligand 2

The gene set enrichment analysis showed that three of these genes are significantly active in 14 biological pathways ( $FDR \leq 0.05$ ), the pathways were checked from the KEGG database and the results are detailed in Table 2.

**Table No. 2: Biological pathways and the number of significant genes involved in each pathway; Based on  $FDR \leq 0.05$**

Genes	Number of hits	Names
CXCL2, CCL20	2	IL-17 signaling pathway
CXCL2, CCL20	2	TNF signaling pathway
CXCL2, CCL20	2	Chemokine signaling pathway
CXCL2, CCL20	2	Cytokine-cytokine receptor interaction
CYP1A1	1	Tryptophan metabolism
CYP1A1	1	Ovarian steroidogenesis
CXCL2	1	Legionellosis
CYP1A1	1	Steroid hormone biosynthesis
CYP1A1	1	Retinol metabolism
CYP1A1	1	Metabolism of xenobiotics by cytochrome P450
CYP1A1	1	Chemical carcinogenesis
CXCL2	1	Salmonella infection
CCL20	1	Rheumatoid arthritis
CXCL2	1	NF-kappa B signaling pathway

**Keywords: *Lactobacillus Acidophilus* -Probiotics-PCA (Principal Component Analysis)-GEO**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Study on the efficiency of fermentation of Nile tilapia fish by-product (*Oreochromis niloticus*) with *Bacillus licheniformis* on the molecular weight profile of the produced proteins

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Nile tilapia is the third most produced fish. Due to the increasing demand for the production of tilapia fish fillet, a large amount of by-products including skin, intestine and viscera, head and tail are produced and causes many environmental problems. The enzymatic hydrolysis, chemical synthesis, and fermentation are the most common methods of hydrolyzing protein to produce bioactive peptides. The purpose of this research is to recover proteins from the by-products of tilapia culture using fermentation and convert them into high-value added products such as bioactive peptides.

#### MATERIALS AND METHODS

Nile tilapia by-products was dried at 55°C for 2 days. The culture medium containing 1% glucose, 0.5% salt, and 10% fish was prepared. After autoclaving, it was inoculated with 5% *B. licheniformis* (IBRCM10204) (v/v) and incubated for 72 hours. The supernatant solution was separated by centrifugation (5000 rpm, 5 min) and the dissolved protein was measured by Bradford, TLC, and SDS-PAGE methods.

#### RESULTS AND DISCUSSION

Bradford's assay showed that the amount of soluble protein has a decreasing trend after bacterial culture. Analysis of soluble protein at different times of fermentation with paper chromatography (TLC) showed that the proteins were hydrolyzed and broken into smaller peptides compared to zero time, which was also confirmed by the protein profile obtained from silver nitrate staining of SDS-PAGE gel. Similar results were reported on by-products fermentation of other aquatic species in literature.

#### CONCLUSION

Fermentation of low-value protein feeds for the production of bioactive peptides using proteolytic microorganisms is an inexpensive alternative to enzymatic and chemical processes.

**Keywords:** Nile tilapia fish by-products, Fermentation, Bioactive peptide, *Bacillus licheniformis*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibacterial and Antibiofilm activity of Diclofenac sodium in combination with Common Antibiotics against multidrug-resistant *Pseudomonas aeruginosa*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

The increasing prevalence of multidrug-resistant (MDR) bacteria is a threat to public health, and the treatment of infections caused by them is associated with serious challenges. However, the combined use of antibiotics and non-antibiotic drugs could be beneficial in the treatment of MDR bacterial infection.

### MATERIALS AND METHODS

In this study, a total of 10 *P. aeruginosa* clinical isolates were investigated. Kirby Bauer Disk diffusion method was used to determine the antibiotic susceptibility of the isolates. The minimal inhibitory concentrations (MICs), minimal biofilm inhibitory concentrations (MBICs), and minimum biofilm eradication concentrations (MBECs) of ciprofloxacin (CIP), meropenem (MEM), cefepime (FEP) and gentamicin (GEN) with/without sub-MICs of diclofenac sodium (DIC; 500, 1000, 2000 µg/mL) for the clinical isolates were determined by the microbroth dilution and microtiter assay methods. the beginning of the

### RESULTS AND DISSCUSION

DIC significantly decreased the levels of MIC to FEP, MEM, and GEN in 2000 µg/mL, also the level of MBICs to FEP, GEN, CIP, and MEM among *P. aeruginosa* isolates significantly reduced in the presence of 2000 µg/mL of DIC. In this study, no significant changes were observed in the MBC level in the presence of diclofenac.

### CONCLUSION

Our study suggests that DIC could increase the efficacy of antibiotics against clinical isolates of *P. aeruginosa* and probably interferes with initial stages in biofilm formation although more investigation is needed.

**Keywords: Antibacterial, Antibiofilm, Diclofenac sodium**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Investigating the effect of Alginate acid nanoparticles and montanide and freond adjuvants the efficacy of a recombinant vaccine candidate against Shigella

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#### ABSTRACT

Shigellosis, an acute intestinal infection, is one of the global health problems caused by Shigella pathogenic species in humans. Production of a cost-effective and protective vaccine against pathogenic species of this bacterium can have a significant effect on public health. The purpose of this research is to express a recombinant chimeric protein as a recombinant immunogen candidate against Shigella pathogenic species and to investigate its immunogenicity with Freund and Montanide adjuvants and protein loaded in allergenic nanoparticles. For this purpose, the gene encoding chimera protein was subhomogenized in pET32 $\alpha$ (+) vector. The correctness of the target gene subisolation was confirmed using PCR reaction. The recombinant vector pET32 $\alpha$ (+) containing the desired gene fragment was transferred into the expression host E.coli BL21 (DE3) and the expression of the recombinant protein was induced using IPTG. The target was purified by urea gradient method. Western blotting method was used to confirm the expression of the recombinant protein. Finally, the purified protein was administered orally and subcutaneously to mice in four groups (combined with montanide adjuvant, Freund's adjuvant, loaded in allergenate gel and control group) on four occasions. Antibody titer against recombinant protein with Montanide and Freund adjuvants and allergenate gel in the serum of mice immunized subcutaneously was 1/3200 and immunized orally by allergenate gel loaded with recombinant protein was 1/1600. Finally, the evaluation of Shigella flexneri neutralization test using antibody produced by immunized animals showed that the serum of immunized animals is capable of inactivating Shigella flexneri. In general, the results of this research show that this recombinant protein is multi-epitope due to having the main epitopes of common antigens between Shigella pathogenic species and also due to the appropriate titer produced against this protein in combination with adjuvants. Freund and Montanide and loading allergens in the gel and the neutralizing power of the resulting antibodies can be a suitable vaccine candidate for the prevention of shigellosis.

**Keywords:** Shigella, shiglose, recombinant protein, multi-VP vaccine, nanoparticles, adjuvant

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# The genome based detection of SARS-CoV-2: Comparison of real-time-PCR and RT-LAMP

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### ABSTRACT

#### BACKGROUND AND OBJECTIVE

On December 30, 2019, the coronavirus appeared and spread extremely rapidly, causing millions of infections and deaths worldwide, and becoming a global pandemic. The most popular method for SARS-CoV-2 detection is genome-based detection such as PCR based that they require expensive experimental equipments, controlled working environment and high-trained technician, which are often lacking in massive viral outbreaks. Therefore, another rapid and simple genome-based assay was evaluated for this pathogen. The aim of this work is to develop a new and sensitive loop test (RT-LAMP) using the E gene sequence for rapid molecular diagnosis of patients, and compare it with the real time RT-PCR method.

#### MATERIALS AND METHODS

For this purpose, 18 samples of patients were collected by medical centers. The total RNA of samples was extracted according to the commercial kit (Roche). Following the initial setup, the RT-LAMP reaction was carried out using specific primers and the performance of the reaction was compared with the real-time PCR method. Also, the positive control construct (TA-E plasmid) was prepared by multiplying the relevant gene fragment and cloning into the TA vector.

#### RESULTS AND DISCUSSION

The sample examined by the real-time-PCR, the golden standard method, is evaluated with 100% conformity with the RT-LAMP method. That result shows the high sensitivity of the RT-LAMP method. It should be noted that in real-time-PCR test cycle threshold (CT) results less than 30 are evaluated as positive. Also, the RT-LAMP assay has a sensitivity of approximately 15 ng and 112 pg for the E gene of SARS-CoV-2 when using extracted total RNA and TA-E plasmid, respectively.

#### CONCLUSION

The RT-LAMP method with equal sensitivity to real-time-PCR is an economical and isothermal method that can compensate for the disadvantages of the PCR method. Overall, this method can be used as a portable, rapid, and easy method for detecting SARS-CoV-2 in the field and clinical laboratories.

**Keywords:** SARS-CoV-2, Genomic diagnosis, RT-LAMP, RT-PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Isolation and identification of grapevine endophytic bacteria and their extracted lipopeptides with antagonistic potential against *Fomitiporia mediterranea*

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

This study aimed to isolate and identify grapevine endophytic bacteria with the ability to produce lipopeptides for antagonistic activity against *Fomitiporia mediterranea*.

### MATERIALS AND METHODS

In this study, a total of 62 bacteria were isolated from grapevine orchards of 12 geographical regions located in Zanjan province. All of the isolates were evaluated in biochemical tests. Six selected strains were identified by nucleotide sequencing of the *16S rRNA* gene. The antifungal effect of isolated strains on four fungal species *i.e.* *Chaetomium globosum*, *Cytospora chrysosperma*, *Fusarium* sp., and *Fomitiporia mediterranea* was done by dual culture method. Also, the methanolic lipopeptide extract of strains extracted and the antagonistic activity of their on *Fomitiporia mediterranea* was investigated.

### RESULTS AND DISCUSSION

The selected strains belonged to known genera including *Bacillus*, *Pantoea*, *Serratia*, and *Variovorax*. In the investigation of the antagonistic activity, 11 isolates showed antifungal properties that two isolates of *Serratia plymuthica* and *Variovorax* sp. showed the most growth inhibitory zones. In addition, the methanolic lipopeptide extract of *Serratia plymuthica* isolate had the highest percentage of inhibition against *Fomitiporia mediterranea*.

### CONCLUSION

Due to the importance of endophytic bacteria and the production of bioactive lipopeptides by them, their isolation and identification from different parts of the country seem necessary, as a result, these bacteria and their lipopeptides extracts can be used in the biological control of plant diseases and can be replaced by chemical control.

**Keywords:** Endophytic bacteria, Methanolic lipopeptide, Antifungal activity, Grapevine

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# Chitosan nanoparticle enhances the efficiency of antimicrobial Radachlorin-mediated photodynamic therapy against *Staphylococcus aureus*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Staphylococcus* sp. displays an important group of Gram-positive bacteria among which a significant resistance against antibiotics is quickly spreading. Antimicrobial photodynamic therapy (aPDT) is a novel strategy to eliminate bacterial infections. In this method by using of a photosensitizer, molecular oxygen, and light of a specific wavelength to excite the photosensitizer, reactive oxygen species (ROS) are produced which lead to microbial cell death. Radachlorin is a natural photosensitizer which is a mixture of sodium salts of chlorin e6, chlorin p6 and purpurin 5 with a high quantum yield of the singlet oxygen production upon red light excitation. The incorporation of Radachlorin into polymeric nanoparticles is considered as a promising strategy for enhancing its aPDT potency. Therefore, the objective of this work was to preparation of biocompatible chitosan nanoparticles with the inclusion of Radachlorin and an evaluation of the possibility of their use for aPDT against *Staphylococcus aureus* ATCC6538 as a model of Gram-positive bacteria.

### MATERIALS AND METHODS

Radachlorin-containing chitosan nanoparticles (Ra-Ch-NPs) were prepared using ionotropic gelation method and was then characterized using Fourier Transform Infrared spectroscopy, UV-vis spectroscopy, scanning electron microscopy, and dynamic light scattering. For PDT, bacterial suspensions of *Staphylococcus aureus* were exposed to either free Radachlorin or Ra-Ch-NPs at different concentrations for 1 h and then both two groups were irritated with red light (640 nm and 20 J/cm<sup>2</sup>). The control groups were exposed to red light alone, Radachlorin alone, and or neither Radachlorin nor light irradiation. Then well diffusion method and counting of colony forming units (CFU) were used to determine the minimum inhibitory concentrations (MIC) of the samples and antibacterial activities in each subgroup. DPBF assay was performed to determine the generation of singlet oxygen after light irradiation. The cytotoxicity of compounds was assessed on normal fibroblast cells using MTT assay.

### RESULTS AND DISCUSSION

The Ra-Ch-NPs had an average diameter of 347 ± 17 nm and zeta potential of +32.4 ± 3.0 mV. The results of aPDT showed that the dark photosensitizer incubation alone,

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as well as using red light irradiation alone did not have a significant antibacterial effect, while irradiation of *Staphylococcus aureus* cells cultured with Ra-Ch-NPs and Radachlorin, significantly induced cell death and colony growth inhibition with inhibition zones  $23 \pm 1$ , and  $16 \pm 2$  mm, respectively. MIC values for Radachlorin and Ra-Ch-NPs after illumination were  $600 \mu\text{g} / \text{mL}$  and  $200 \mu\text{g} / \text{mL}$ , respectively.

Comparatively, Ra-Ch-NPs indicated higher photodynamic inactivation than free Radachlorin with 4.7 and 3.2  $\log_{10}$  (CFU/mL) reductions in *Staphylococcus aureus*, respectively. The Radachlorin ( $46.06 \pm 2.23\%$ ) and Ra-Ch-NPs ( $51.14 \pm 1.44\%$ ) displayed remarkable singlet oxygen production which was comparable to methylene blue ( $55.36 \pm 0.27\%$ ). None of the tested compounds either in the absence of light or after light irradiation showed toxicity on normal fibroblast cells.

### CONCLUSION

Considering the results of this work, aPDT with Ra-Ch-NPs and Radachlorin is effective in killing *Staphylococcus aureus*, although Ra-Ch-NPs are more efficient than free Radachlorin. It will be intriguing to affirm these data by further in vivo researches.

**Keywords:** Photodynamic therapy, *Staphylococcus aureus*, Radachlorin, Chitosan, Nanoparticle

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Identification of some specific genes in *Campylobacter jejuni* associated with the response to acidic pH by using bioinformatics analysis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Campylobacter jejuni* is one of the leading causes of bacterial gastroenteritis and the most frequent cause of campylobacteriosis in the globe. This bacterium can have negative consequences and diseases like sepsis, meningitis, urinary tract infections, hemolytic uremic syndrome in people, and ailments in birds anywhere in the world. Due to the rapid rise of multidrug-resistant isolates, *C. jejuni* is categorized as a member of the pathogenic group. The flesh of birds and other animals is the most popular means of transmission to humans. It can be very helpful to understand how to regulate the diseases this bacterium causes by looking at how its genes are expressed under stress situations like an acidic pH. This was accomplished in this study using bioinformatics methods.

### MATERIALS AND METHODS

GEO2R web tools and R software are used to locate, extract, and analyze the microarray data with the accession ID GSE9937. Using the criteria  $P < 0.05$  and  $\text{LogFC} > |1|$ , the genes with the greatest differential expression were found. After isolating the expression of the associated genes, the protein network for the genes that had increased expression was predicted by the STRING database and visualized using the Cytoscape program.

### RESULTS AND DISCUSSION

1653 genes were obtained as DEGs (889 upregulate, 764 downregulate). Our analysis revealed 5 hub genes, namely, *secY* (Sec export pathway or Sec-dependent pathway), *cj0414* (gluconate metabolism), *acnB* (aconitate hydratase B), *cj0145* (Twin-Arginine Translocation), *ribD* (riboflavin synthesis). Furthermore, the results of the KEGG pathway analysis revealed that these genes *C. jejuni* bacteria play a major role in pathogenesis.

### CONCLUSION

Overall, in this study we identified differentially expressed genes in *C. jejuni* in response to acidic pH through bioinformatics analysis of gene expression data. Identifying these genes provides valuable insights into the molecular pathways that *C. jejuni* employs to maintain its viability, respond to environmental stressors and cause infections and aid in the identify development of more effective control measures for combating infections and designing preventive strategies.

**Keywords:** *Campylobacter jejuni*, Acidic pH, Pathogenesis, Gene expression analysis, bioinformatics.

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# The effect of ferulic acid encapsulated chitosan on methicillin-resistant *Staphylococcus aureus*

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Increasing antibiotic resistance and diminishing pharmaceutical industry investments have increased the need for molecules that can treat infections caused by dangerous pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA). Ferulic acid shows tremendous therapeutic properties with potent antibacterial properties. Among the nanomaterials used for efficient delivery of drugs, chitosan has received emerging interest due to its physical and chemical properties. Besides, its combination with tripolyphosphate (TPP) not only enhances the entrapment efficiency of the drug but also results in controlled and targeted delivery. In this study, ferulic acid encapsulated chitosan-tripolyphosphate nanoparticles (FANPs) was investigated against methicillin-resistant *Staphylococcus aureus* (MRSA) and compared with native ferulic acid.

### MATERIALS AND METHODS

#### Synthesis of ferulic acid encapsulated chitosan-TPP nanoparticles (FANPs)

FANPs were synthesised by the ionic gelation method. Briefly, ferulic acid was added gradually into chitosan solution (0.25% w/v) under magnetic stirring at ambient temperature for 30–45 min followed by dropwise addition of 0.25 mg/ml of TPP solution in a ratio of 1:5 with respect to chitosan under continuous stirring at 1000 rpm for 30 min to yield FANPs. The suspension of nanoparticles was then centrifuged at 12,000 rpm for 60 min and washed with deionised water (three times) to remove the unloaded compounds.

#### Physicochemical characterisation of synthesised FANPs

Dynamic light scattering (DLS) was used to measure the mean particle size of synthesised FANPs using particle size analyser (Malvern-Nano Series) at 25°C with scattering angle of 90°.

#### Fourier transform infrared (FTIR) analysis

FTIR spectra of chitosan, ferulic acid and FANPs were recorded to analyse the bonds and functional groups present.

#### Determination of minimum inhibitory concentration (MIC)

The MIC for the native ferulic acid and FANPs were determined as per the guidelines of Clinical and Laboratory Standards Institute.

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### RESULTS AND DISCUSSION

#### Synthesis of FANPs

Ferulic acid was encapsulated into chitosan biopolymer where TPP was used as a crosslinker, in order to synthesise FANPs.

#### Physico-chemical characterisation of synthesised FANPs

The DLS analysis confirmed the formation and size distribution of FANPs. The size of FANPs, analysed by DLS showed a Z-average value of 215.55 nm.

#### FTIR spectrophotometer analysis

In order to confirm the successful formation of FANPs, FTIR studies were carried out. The presence of similar kind of functional group in FANPs in comparison to native ferulic acid and chitosan signifies that ferulic acid was successfully encapsulated into chitosan carrier where TPP act as crosslinker.

#### Determination of MIC and sub-MIC

The MIC of ferulic acid and FANPs against methicillin-resistant *Staphylococcus aureus* (MRSA) was found to be 1000 µg/ml where the growth and cell density were significantly inhibited as evident from the absence of turbidity. Hence, sub-MIC concentrations of 250 and 500 µg/ml (i.e. 1/2 and 1/4 MIC, respectively) were used for further activities.

### CONCLUSION

This study showed as compared to native ferulic acid, the attenuation efficacy of FANPs was found to be significantly higher suggesting the role of a biocompatible nanocarrier system for effective targeting of the virulence with slow and controlled release of encapsulated drugs.

**Keywords:** MRSA, FANPs, DLS, MIC

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Identification of *bla*<sub>NDM-1</sub>, virulence factors, and plasmid replicon types in Carbapenem-Resistant *Salmonella* spp. in Kerman, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Salmonella* species (spp) are the most prevalent zoonotic pathogens that cause outbreaks of gastroenteritis in the world, so evaluation of the profile of antibiotic resistance and the potential pathogenicity of these bacteria is necessary to prevent the spread of potentially pathogenic and drug-resistant strains.

### MATERIALS AND METHODS

In this study, 39 *Salmonella* spp. were isolated from fecal samples of the chicken farm and the antibacterial susceptibility of isolates was determined by disk diffusion.  $\beta$ -lactamases (*bla*) including ESBLs, AmpC, MBLs, and virulence genes were detected by PCR and plasmid incompatibility groups of the isolates were identified by using PCR-based replicon typing (PBRT).

### RESULTS AND DISCUSSION

The most prevalent virulent gene was *phoP/Q* (84.6%) and *slyA*, *sopB*, and *stn* were identified in 79.4% (n=31), 69.2% (n=27), and 2.5% (n=1) of the isolates, respectively. The antibiotic susceptibility testing showed that 30.7% of the isolates were ESBL-producing. *bla*<sub>TEM</sub> (41%; n=16) was the most  $\beta$ -lactamases gene among the isolates and followed by *bla*<sub>NDM-1</sub> (15.4%; n=6), *bla*<sub>DHA</sub> (7.7%; n=3), and *bla*<sub>CTX-M</sub> (1.5%; n=1). Six different replicon types, including IncP (n=9; 23%), IncFIC (n=3; 7.70%), IncY (n=3; 7.70%), IncI1-I $\gamma$  (n=2; 5.12%), IncFIIAs (n=1; 2.56%), and IncN (n=1; 2.56%) were identified using PBRT method.

### CONCLUSION

Our study showed the emergence of carbapenem-resistant and *bla*<sub>NDM-1</sub> among *Salmonella* spp. for the first time in Kerman, Iran. Since *Salmonella* spp. plays an important role in the transmission of resistance genes in livestock and humans in the food chain; more stringent control policies are recommended to prevent the circulation of drug-resistant and potentially pathogenic strains from animals to humans.

**Keywords:** *Salmonella* spp., *bla*<sub>NDM-1</sub>, Virulence, Plasmid replicon types

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The identification of genes related to salinity stress tolerance in *Pseudomonas* sp. SWRIQ11 (CECT 30741)

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

Identification of new halotolerant bacteria taxa with special beneficial features is increasingly needed. In this research the genome of SWRIQ11 is assessed.

#### MATERIALS AND METHODS

The whole genome sequencing was carried out by the Illumina HiSeq platform. The gene detection and genome annotation were done exploiting the Rapid Annotation using Subsystem Technology (RAST) server, NCBI Prokaryotic Genome Annotation Pipeline (PGAP), KEGG Automatic Annotation Server (KAAS), and UniProt database. Gene clusters related to secondary metabolites biosynthesis were determined using antiSMASH.

#### RESULTS AND DISCUSSION

The strain SWRIQ11 can tolerate salinity up to 6% w/v NaCl. The SWRIQ11 inoculation did not induce necrosis in leaves in the hypersensitive response (HR) assay, and as a result, the strain is not considered phytopathogenic. Moreover, the strain had no hemolytic activity. The genome of the strain SWRIQ11 contained an assembly size of 6,196,390 bp with GC content of 60.1%. The genome of SWRIQ11 was rich in genes related to stress response sigma factors, stress sensors, signaling and regulation proteins. Moreover, genes related to main adaptation mechanisms for tolerating salinity stress including synthesis of chaperons, exopolysaccharides, and osmoprotectants, ion homeostasis, nutrient acquisition, and antioxidants defenses were identified in the genome of SWRIQ11. The results of genome analysis are in accordance with laboratory experiments and *Pseudomonas* sp. SWRIQ11 is a halotolerant rhizobacteria. The genome of *Pseudomonas* sp. SWRIQ11 contained the genes related to plant growth promotion, biocontrol agents, antitumor and herbicide capabilities, and biodegradation.

#### CONCLUSION

In conclusion, *Pseudomonas* sp. SWRIQ11 is a potential safe halotolerant strain with different efficiencies like biocontrol, antitumor, herbicide, and biodegradation capabilities which must be further assessed.

**Keywords:** salinity stress, halotolerant bacteria, *Pseudomonas* sp., genome analysis, genes and pathways

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# The cryoprotective effect of egg yolk and polyvinylpyrrolidone on the survival of *Lactobacillus plantarum* during lyophilization

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### ABSTRACT

As the demand for probiotic supplements increases, so does the need for high-quality probiotics and their long-term preservation. *Lactobacillus plantarum* is one of the most useful probiotic bacteria that are widely used in fermentation products and probiotic supplements. To preserve *Lactobacillus plantarum* powders in good condition, a variety of maintenance approaches have been developed and used. One of these methods is freeze drying. In the biological sciences, freeze drying is a well-known technique. The cell is damaged throughout the freezing and drying process, which is one of the major drawbacks of this technique. Various cryoprotectants have been developed and utilized to reduce this damage. In the first phase of this study the cryoprotectant efficacy of egg yolk and polyvinyl pyrrolidone hydrogel, as well as their composition in various percentages, were studied on *Lactobacillus plantarum*. The best sample from the first phase was selected, and it was combined with skim milk in various percentages in the second phase of the experiment, and the effect was analyzed. Statistical analysis was performed by the SPSS method. The highest survival effect was obtained with skim milk (SR=95/2%). SEM images of cryoprotectant surfaces were acquired.

### CONCLUSION

The main evaluation criterion of this study was the statistical analysis. As seen in the statistical figure 3-1, in the first phase, sample number 2 has the highest survival rate in the first phase. The control sample, which had no cryopreservation, had the lowest survival rate. Each substance's impact was nearly equivalent and similar to one another. The best result is obtained when PVP is mixed with egg yolk and the amount of egg yolk is less than the amount of PVP. Sample 3, in which each ingredient was used equally, was particularly noteworthy. This indicates that these Substances do not overlap well in equal proportions. It can be stated that both egg yolk and PVP have been shown to considerably protect *Lactobacillus plantarum* from FD damage. But Skim milk performed significantly better than the OS in the second stage. Skim milk is a combination of simple carbohydrates and protein and the amount of fat in it is too low. But egg yolk has a high amount of fat, but its carbohydrate and protein levels are lower than skim milk. PVP is a chemical that is made by strong hydrogen bonds with phenolic, hydroxyl, and carboxyl groups. It has previously been proven that compounds containing proteins and carbohydrates have a higher cryoprotective effect. In this study, skim milk was selected as the best ingredient for a cryoprotectant during FD for the *Lactobacillus plantum* bacteria.

**Keywords:** Probiotic, *Lactobacillus plantarum*, Freeze-drying, Lyophilization, Cryoprotectant, Egg yolk, Polyvinyl pyrrolidone, Skim milk

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Altering Gut-Associated Microbiota: A Possible Role in the Pathogenesis of Ulcerative Colitis

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Gastrointestinal microbiota are closely related to the pathogenesis of ulcerative colitis (UC). In this study, the relative abundance of microbial populations between the UC and non-UC subjects were evaluated by quantitative real-time polymerase chain reaction (qRT-PCR).

### MATERIALS AND METHODS

DNA extraction from biopsies and polymerase chain reaction (PCR) amplification of bacterial 16S rRNA gene-targeted species-specific primers was performed to detect the anaerobic bacterial species. The qRT-PCR was used to show the relative change in the bacterial populations of *F. prausnitzii*, *Provetella*, and *Peptostreptococcus* in the UC and non-UC subjects.

### RESULTS AND DISCUSSION

Our data for detection of the anaerobic intestinal flora showed *Faecalibacterium prausnitzii*, *Provetella* and *Peptostreptococcus* were the predominant microflora in the controls and showed significant differences ( $p = 0.002$ ,  $0.025$  and  $0.039$ , respectively). The qRT-PCR analyses of *F. prausnitzii*, *Provetella* and *Peptostreptococcus* were 8.69-, 9.38- and 5.77-higher, respectively, in the control group than in the UC group.

### CONCLUSION

The decreased abundance of *F. prausnitzii*, *Provetella* and *Peptostreptococcus* in the intestine may decrease anti-inflammatory activity in the mucosa, biosynthesis of vitamins and the ability to degrade plant polysaccharides and digest carbohydrate-rich food. Additionally, the decreased ability of microbes to utilize mucins and metabolize tryptophan could reduce protection of the gut mucosa and be relevant to the pathophysiology of UC.

**Keywords:** Ulcerative colitis, Microbiota, Quantitative real-time PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of the antibacterial effect of carvacrol alone and in combination with the antibiotic cefixime against *Escherichia coli* O<sub>157</sub>:H<sub>7</sub>

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Today, the use of plant compounds and their derivatives such as extracts and essential oils to combat infectious agents is highly regarded by researchers. One of the active antimicrobial compounds with plant origin is carvacrol. The aim of the present study was to evaluate the antibacterial activity of carvacrol alone and in combination with the antibiotic cefixime against *Escherichia coli* O<sub>157</sub>:H<sub>7</sub>.

### MATERIALS AND METHODS

The Antibacterial properties of carvacrol and cefixime were evaluated by determining the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and disk diffusion method. The Checkerboard assay was used to evaluate the interaction between the carvacrol and cefixime and to determine the fractional inhibitory concentration.

### RESULTS AND DISCUSSION

The result showed that the MIC and MBC of carvacrol and cefixime against *E.coli* O<sub>157</sub>:H<sub>7</sub> was 250, 250 µg/ml (MIC, MBC) and 128, 128 µg/ml (MIC, MBC) Respectively. In the checkerboard test, carvacrol had synergistic interaction with antibiotic cefixime against *E. coli* O<sub>157</sub>:H<sub>7</sub> (FIC index=0/5).

### CONCLUSION

Due to the significant antibacterial activity of carvacrol, the present study introduces this agent as a new antibacterial drug with natural origin. In addition, since carvacrol significantly increased the antibacterial potential of cefixime (synergistic properties), carvacrol could be introduced as an effective compound to increase the antibacterial power of cefixime antibiotic.

**Keywords:** *Escherichia coli* O<sub>157</sub>:H<sub>7</sub>, carvacrol, cefixime, Antibacterial, checkerboard

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Antiviral Potential of probiotics

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Probiotics are live microorganisms that offer a range of health benefits. The use of probiotics is widespread in managing various diseases, including gastrointestinal, respiratory, and urinary tract infections. With the rise in viral infections, and by growing need to investigate the antiviral properties of probiotics to prevent or manage viral infections.

#### MATERIALS AND METHODS

the mechanisms that contribute to their beneficial effects, including the production of antimicrobial substances, regulation of immune and inflammatory responses, stimulation of mucus secretion, and the maturation of dendritic cells. Some specific strains of probiotics have been found to increase levels of pro-inflammatory cytokines and reduce symptoms of viral-disease, Probiotics also exhibit antiviral activity by impeding virus adsorption.

#### RESULTS AND DISCUSSION

With the rise in viral infections, growing need to investigate the antiviral properties of probiotics to prevent or manage viral infections; *Lactobacillus acidophilus* has been shown to improve innate immunity, increase antiviral cytokine production, and inhibit virus adhesion and replication. *Bifidobacterium bifidum* enhances the production of antibodies against viruses, regulates the immune response, inhibits viral replication. *Lactobacillus rhamnosus GG* stimulates the immune system, reduces the severity of viral infections, and inhibits the replication of pathogenic viruses, *Saccharomyces boulardii* stimulate the body's immune response against viral infections and reduce the duration and severity of symptoms.

#### CONCLUSION

the antiviral properties of probiotics are strain-specific and depend on various factors such as the mode of action, the type of virus, and the host's immune response. the antiviral effect is likely achieved through direct physical interaction between bacteria and virus particles, which impairs virus entry into its mammalian host cell the antiviral activities of probiotics are limited to certain viruses, it targets viral proteins to inhibit the multiplication of the virus This could be achieved via the inhibition of viral entry and replication, or by suppression of the immunologic response that is provoked by the infection (known as the cytokine storm), although peptides like p18 extracted from probiotics, which can have antiviral properties. The antiviral properties of probiotics provide a promising approach to managing and preventing viral infections.

**Keywords: Probiotics, Antiviral, Health-Promoting, Immune System**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Stability Evaluation of Ring Antigen Produced by RVSRI

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The Milk Ring Test (MRT) is valuable and useful in detecting *Brucella* antibody in milk. The evaluation of the stability of this antigen is used to confirm its effectiveness and its expiration date. It plays an important role in ensuring the quality of the product. Therefore, it is necessary to monitor the antigen ring test produced by Razi Vaccine and Serum Research Institute (RVSRI) and in order to determine the maximum shelf life of the antigen ring test.

#### MATERIALS AND METHODS

100 sample vials of three series of antigen produced by RVSRI were performed in accelerated and long term stability methods. In the accelerated stability studies, the products were examined at a temperature of 37 degrees for a period of up to 3 months, which is approximately equivalent to three years. Another method is to examine long-term stability studies, where the vaccine is placed in the recommended storage conditions of +4 to 36 months and +25 to 18 months. The following tests were performed including inactivity of *Brucella abortus* bacteria, the amount of mass per unit volume, the amount of phenol, pH level, purity and identity, the positive and negative control of the antigen.

#### RESULTS AND DISCUSSION

The results of the long-term stability studies showed that the antigen was confirmed and in accordance with the OIE standard. All the samples had similar results to the samples tested in the long-term evaluation method. This study showed that the MRT antigen produced by RVSRI was within the standard range in the time frame of the performed tests (2 and a half years), which lasts up to three years of study.

#### CONCLUSION

Based on years of experience, all the antigens produced by Razi Vaccine and Serum Research Institute can be used for years provided that they are kept in proper storage conditions (2 to 8°C) and that autoagglutination in the antigen has not been occurred.

**Keywords:** Antigen, Milk Ring Test, Stability, Brucellosis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Cloning and expression of the *Brucella melitensis* OMP28 and OMP31 genes in *Lactococcus Lactis* and investigations of immunogenic responses in BALB/c Mice

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Brucellosis is a common disease between human and animals in the world and the disease imposes a huge costs on the economy and society. There is still no suitable vaccine against this disease. Currently, the Live Brucella vaccines have disadvantages such as the inability to differentiate infected animals from vaccinated with classical Brucella tests in the first year, antibiotic resistance, the possibility of shedding the vaccine strain from milk and other animal secretions and the possibility of abortion of vaccinated animals. Finally, the virulence potential of common vaccine strains for humans led to the evaluation of the OMP31 and OMP28 recombinant proteins and their immunogenicity in the prevention of the disease.

### MATERIALS AND METHODS

In this study, OMP28 and OMP31 recombinant proteins were isolated from *Brucella melitensis* and cloned in *Lactococcus lactis* NZ3900 vector. Colonies that have received the vector will turn yellow on this medium. After transformation of the genes to the host and the expression of the recombinant protein, mice were given the bacteria as an oral vaccines. Evaluation of the immunogenicity of the vaccine was performed orally in mice (2-5 x 10<sup>9</sup> cells for each mouse). Serum samples were collected from all mice on days 0, 7, 14, 21, 28 and antibody levels were measured against OMP28 by ELISA method.

### RESULTS AND DISCUSSION

The results showed a significant increase in the antibody titer of the vaccinated groups against OMP28 and OMP31 compared to the control group. It should be noted that all rats were weighed before the test and quarantined for two weeks for environmental compatibility in the laboratory.

### CONCLUSION

All these attempts to express this protein and investigate its antigenicity, indicating the high antigenicity of protein derived from OMP31 and OMP28 gene expression.

**Keywords:** Expression, OMP, Cloning, *Brucella Melitensis*, Immunogenicity.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Molecular Survey of *Brucella melitensis* Field Isolates using Sequence-Based PCR of OMP31

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

Brucellosis is a disease that imposes huge costs on the economy and society. It is one of the common diseases between humans and livestock in the world. Sequencing-based molecular methods have been introduced as an effective and repetitive method for bacterial strain typing, which can provide a reliable typing approach for clinical laboratories. The aim of this study is to describe the reproducibility and performance of the Outer Membrane Protein 31 (OMP31) based PCR, as a molecular genotyping tool for *Brucella melitensis* typing.

#### MATERIALS AND METHODS

In this study, 146 samples were taken from human blood samples, bovine and camel lymph nodes, as well as sheep and goat aborted fetuses, including fetal kidney, abomasum, liver, lung, spleen, and heart for bacteriological investigation. The molecular detection of the *Omp31* and *IS711* genes was performed using the isolated *B. melitensis* (n=14). The sequencing of the *Omp31* gene of *B. melitensis* in the Iranian field isolates was also performed for the whole gene sequencing. The homology of all sequences was then checked with the reported National Center for Biotechnology Information sequences using a basic local alignment search tool for the nucleotide diversity evaluation.

#### RESULTS AND DISCUSSION

The results showed that *Brucella melitensis* isolates were recovered from 14 examined cases and confirmed by IS711-based PCR method with PCR product of 731 bp. The 14 *Omp31* gene sequences were clustered as a single branch group with bootstrap support of 63, and they were closely correlated to the *Brucella melitensis* reference isolates which was determined in NCBI database.

#### CONCLUSION

Phylogenetic analysis based on OMP31 in animal and human hosts showed genomic similarity in isolates of different origins. Sequencing of this gene can also be used as a tool for identification of *Brucella melitensis* species and genus.

**Keywords:** OMP31, *Brucella Melitensis*, Phylogenetic analysis, Sequence-based PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Serological Responses of *Brucella Melitensis* Vaccine Rev-1 Strain in Lambs

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Brucellosis is a common infectious disease between humans and livestock and is still a major problem in most parts of the world. *Brucella melitensis* Rev-1 vaccine is one of the effective vaccines for preventing and controlling brucellosis in young lambs. In this study, serological responses of Razi institute's *Brucella* Rev-1 vaccine and its comparison with the commercial Spanish vaccine (CZV) were evaluated.

### MATERIALS AND METHODS

12 female sheep (5-8 months old) free of brucellosis were divided into 3 groups of 4. One group was injected as a control by injection of physiological serum and the other two groups with *Brucella melitensis* vaccine made by Razi Institute and Spanish *Brucella melitensis* (CZV) vaccine, respectively. Blood samples and sera were collected from all groups in 0, 2, 4 and 6 weeks after vaccination. All animals with brucellosis strain 16M were challenged after six weeks of vaccination. Then, all animal serum samples were evaluated by Rose Bengal (RBPT), Wright (SAT) and 2ME (2-Mercaptoethanol) and ELISA assay.

### RESULTS AND DISCUSSION

The results showed that the animals in the control group were serologically negative in all experiments during the 6 weeks before the challenge experiment. The first positive serologic reaction based on RBPT, SAT and 2ME test results was recorded in the second week in both groups and was positive up to 6 weeks after vaccination. In the fourth week, agglutination titer increased in both groups vaccinated with domestic or commercial CZV vaccine and there was no significant difference between both groups with serologic results of SAT and 2ME ( $P > 0.05$ ). The results of the challenge experiment showed that all vaccinated and control groups showed positive serological responses at weeks 2, 4 and 6 with increased agglutination titer.

### CONCLUSION

The positive serological reactions of the vaccine group (made by Razi Institute) were similar to the positive reactions in the commercial vaccine (CZV) group. It seems that due to similar results of two vaccines in stimulating the humoral immune system of young lambs affect the function of CD4 Th2 type lymphocytes and are currently under investigation.

**Keywords:** Vaccine, *Brucella melitensis*, Serology.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Evaluation of different concentrations of thionin and basic fuchsin media for typing of *Brucella* spp. biovars

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Brucellosis, “undulant fever”, is an infectious disease caused by the bacterial genus *Brucella*. Health management and timely diagnosis of brucellosis are of great importance for any effective control strategy in humans and animals. This study was aimed at investigating the appropriate concentrations of thionin and fuchsin dyes to identify the biovars of this bacterium.

### MATERIALS AND METHODS

A concentration of 0.4% of thionin and fuchsin dyes in distilled water, as well as serum dextrose, tryptic soy agar and Brucell agar were employed as basic media for making colored media.

Thionin and basic fuchsin solutions were boiled in a bain-marie for one hour. Then concentrations of 10, 20, and 40 micrograms/ml for thionin dye and 10 and 20 micrograms/ml for fuchsin dye were made based on OIE instructions. Furthermore, the concentration of 0.1% dyes in the 2.5, 5, and 10 µg/ml for thionin dye, and 5 and 10 µg/ml for fuchsin were used for comparison. Horse serum was also considered as a factor stimulating the growth of *Brucella* bacteria in environments.

### RESULTS AND DISCUSSION

The findings presented in our study have demonstrated that a selective culture medium of *Brucella* agar along with serum is the best option for making colored media in the differentiation of *Brucella* biovars in classical typing. The percentage of dyes applied in colored media at the rate of 10, 20, and 40 µg/ml for thionin dye and 10 and 20 µg/ml fuchsin dye showed the best accuracy in differentiating different *Brucella* biovars. By increasing sterile and inactivated horse serum by 5%, bacterial growth increased compared to serum-free environments.

### CONCLUSION

Although serum dextrose, tryptic soy agar media have been used as basic media for making colored media in the past, the selective culture media of *Brucella* agar with horse serum, showed a high sensitivity for the growth of *Brucella* biovars, leading to better growth of *Brucella*.

**Keywords:** Thionin, Fuchsin, Typing, Biovar, *Brucella*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antimicrobial Effect of *Lavandula* and *Rosemary* Extracts on *Staphylococcus aureus*: In vitro and Animal Model

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Infectious diseases are one of the most common diseases around the world which impose enormous financial burden on society. *Staphylococcus aureus* is an important cause of nosocomial infections and multidrug resistance. Although synthetic antibiotics have been able to play an important role in treatment of infectious diseases in past decades, however problems related to microbial resistance of antibiotics have caused that the medical plants to be considered as an alternative.

### MATERIALS AND METHODS

Ethanollic and acetonic extracts were prepared from dried leaves of the *Lavandula angustifolia* and *Rosmarinus officinalis*, then anti-bacterial activities of the extracts for *Staphylococcus aureus* were experimented, first by the method of well diffusion in agar, and later the amount of the MIC and MBC of the extracts were measured by broth microdilution method. In animal model, first  $5 \times 10^5$  CFU/ml of bacteria was intraperitoneally injected and after 24 hours, 0.5ml (as MBC concentration of each the extracts) of extracts, to female BALB/c mice was intraperitoneally injected. Then, the counting of bacterial colonies in spleen were determined with cultivation on Mueller Hinton agar after 7 days as the standard protocol.

### RESULTS AND DISCUSSION

The experiment results concerning the determination of growth inhibition diameter in agar showed that the maximum of growth inhibition diameter is related to the acetonic extract of *Lavandula angustifolia* (20 mm), and the minimum of growth inhibition diameter is related to ethanolic extract of *Rosmarinus officinalis* (10 mm) at the highest concentration (400 mg/ml). In conditions of in vivo, after 48 hours spleen supernatant cultivation, the average number of bacteria for acetonic extracts of the *Lavandula angustifolia* and *Rosmarinus officinalis* were  $1.8 \times 10^3$  CFU/ml and  $6.6 \times 10^3$  CFU/ml respectively and for ethanolic extracts of *Lavandula angustifolia* and *Rosmarinus officinalis* were  $10.8 \times 10^3$  CFU/ml and  $14.6 \times 10^3$  CFU/ml respectively. These results showed significantly decrease in number of bacteria in all experimental groups ( $p < 0.5$ ) compared to control group.

### CONCLUSION

In general, the results of evaluations in experimental conditions and the animal model showed that acetonic and ethanolic extracts of these plants have the effective antibacterial activity against mentioned bacteria and can be useful to treatment of nosocomial infections.

**Keywords:** Antimicrobial, *Lavandula angustifolia*, *Rosmarinus officinalis*, *Staphylococcus aureus*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Antibacterial and Healing Effect of Silver Nanoparticles Derived from Nettle Extracts on Burn Infections of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

*S. aureus*, *E. coli* and *P. aeruginosa* are common causes of burn infections and illnesses. Silver nanoparticles have many applications, one of the necessary applications is antimicrobial and healing effect. There are various methods for synthesis like green synthesis which is more efficiency and eco-friendly. In this study, antibacterial and healing effect of silver nanoparticles derived from *Urtica dioica* on burn infections of *S. aureus*, *E. coli* and *P. aeruginosa* has been reported.

### MATERIALS AND METHODS

In this study, acetonc extracts of *Urtica dioica* prepared with soaking method. The extract was added to 0.1 M of silver nitrate solution for biosynthesing of Ag<sup>+</sup> nanoparticles. Efficiency of Ag<sup>+</sup> synthesis was confirmed by XRD and DLS technique. The MBC and MIC of the extracts and nanoparticles were determined with microdilution method. In animal model, bacteria were inoculated with a concentration of (5×10<sup>5</sup> CFU/ml) to the wound side of rats. After 24 hours ointments were prepared based on MBC concentration of extracts and nanoparticles with 1g Eucerin for treat burn wounds and infections.

### RESULTS AND DISCUSSION

Animal studies shows that silver nanoparticles synthesized by *Urtica dioica* are effective on *S. aureus*, *E. coli* and *P. aeruginosa*. But acetonc extract of mentioned plant is effective on *S.aureus* and *E.coli*. As a result, silver nanoparticles derived by extracts have more antimicrobial and healing effect than acetonc extracts. The wound which treated with nanoparticles seen healing activity.

### CONCLUSION

Acetonc extracts of mentioned plant and silver nanoparticles obtained by *Urtica dioica* have antimicrobial and healing activity in low concentration on *S. aureus*, *E. coli* and *P. aeruginosa*. But acetonc extract of *Urtica dioica* have healing and antimicrobial activity in low concentration on *S. aureus*, *E. coli* and effective in high concentration on *P. aeruginosa*.

**Keywords:** *Escherichia coli*, silver, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Urtica dioica*.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# In silico study of *Citrobacter amalonaticus* phytase enzyme to increase thermal stability

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Enzymes are natural nanoscale biocatalysts that catalyze chemical reactions in living cells. Phytase is the most widely used food enzyme in the world. Phytases are a subgroup of phosphatases that are capable of phosphorylating phytate, the most abundant inositol phosphate in nature.

In order to be used in industrial processes, phytases must have high specific activity and the ability to function in the conditions of the digestive system of animals and to withstand short-term exposure to high temperatures during feed granulation (60-80 degrees Celsius). The aim of this study is to increase the thermal stability of phytase enzyme by applying stabilizing mutations.

### MATERIALS AND METHODS

The phytase gene of *Citrobacter amalonaticus* with a sequence of 1311 base pairs, which encodes an enzyme with 436 amino acids, was selected in order to improve stability. This phytase belongs to the family of histidine acid phosphatases. In the first step, the main protein was cloned and its biochemical indicators were investigated. Then, using MUPRO software (<https://mupro.proteomics.ics.uci.edu>), the desired mutations were measured to increase the thermal stability of the enzyme, and 64 mutations were selected among the 990 mutations examined. The selection of amino acids for the mutation was based on the hydropathy index and the amount of presence in the protein. Amino acids glycine, alanine, valine, isoleucine, leucine and serine are suitable candidates for mutation.

### RESULTS AND DISCUSSION

Finally, based on the three-dimensional structure of the protein, after applying each of these 64 mutations using the I-TASSER server, 3 mutations were selected as the final candidates, and the simulation of each of these mutations was done separately, and in terms of biochemical indicators with enzymes The original was compared.

### CONCLUSION

After checking the secondary simulation results, Q190L and T305L mutations were applied. Of course, our work is at the level of prediction and it should be checked experimentally to prove its correctness.

**Keywords:** Phytase, *Citrobacter Amalonaticus*, Increased Thermal Stability, Mutation

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Omega-3 derived from Fish Oil and Probiotics Synergist Effect through Co-Encapsulation: A review

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### ABSTRACT

Functional foods represent a rapidly growing sector in the food industry. One area of emerging research and product development involves the creation of functional foods that combine omega-3 fatty acids and probiotic bacteria through a co-microencapsulation process. This review present relevant literature on various microencapsulation methods, with a specific focus on incorporating probiotic bacteria and omega-3 oils into a single delivery format. The review encompasses information on the benefits and drawbacks of co-encapsulation using three different methods, as well as the bio-accessibility of omega-3 in the human gastrointestinal tract. While co-encapsulation has been extensively utilized in pharmaceutical delivery systems, it is a relatively new concept in stabilizing and delivering food ingredients. There is a commercial demand for co-encapsulation of multiple bioactive ingredients within a single microcapsule due to cost reduction and improved product quality. While in-vitro evaluations provide valuable insights, further in-vivo and clinical trials are necessary to determine the effectiveness of bioactive ingredient release, particularly in microcapsules containing multiple bioactive components.

### CONCLUSION

The technical and commercial feasibility of microencapsulating omega-3 oils and probiotic bacteria for their incorporation into food products has been established. However, it is necessary to conduct tests to assess the compatibility of these microcapsules with various food and beverage items, in order to understand their impact on product shelf-life and sensory characteristics. Currently, there are functional foods available that are fortified with either omega-3 oils or probiotic bacteria, but not both. There is a growing demand in the market for the co-encapsulation of multiple ingredients within a single microcapsule, as it offers cost savings and enhances product quality. Various coating techniques, such as fluidized-bed drying, have demonstrated their effectiveness in co-encapsulating multiple unstable components.

**Keywords: Probiotic, Omega-3, Co-Microencapsulation**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Pattern of macrolide resistance in *Clostridium perfringens* isolates from small ruminants in Kerman, Iran

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Clostridium perfringens* (*C. perfringens*) is an anaerobic spore-forming Gram-positive bacteria, ranking amongst the most important pathogens living in the intestinal tracts of both humans and animals. Its pathogenicity is largely attributed to produce several potent toxin and several extracellular enzymes which can cause histotoxic infections, food poisoning and a spectrum of intestinal diseases like enteritis and enterotoxaemia. Enterotoxaemia is a pathological condition characterized by the absorption of toxins produced within the intestinal tract, which ultimately leads to deleterious effects on several internal organs, including the brain, kidneys, lungs, and heart. It is one of the most frequently reported diseases of sheep and goats all over the world. In different countries, the prevalence rates of the enterotoxaemia ranges from 24.13 to 100% in small ruminants. Antimicrobial agents are used for control and treatment of this disease and sometimes clinical outbreaks do not respond well to certain treatments. The objective of this study was to determine the resistance of *C. perfringens* isolates to macrolide antibiotic class.

#### MATERIALS AND METHODS

This study was performed on a total of 273 *C. perfringens* isolates, which were previously recovered from fecal samples of the diseased small ruminants in Kerman province, Iran. They were evaluated for the antimicrobial resistancy against tylosin and erythromycin (macrolide class) antibiotics by Kirby-Bauer disk diffusion method.

#### RESULTS AND DISCUSSION

The findings of our study demonstrated the high antibiotic resistant rate of *C. perfringens* isolates in small ruminants. The highest resistancy were observed to tylosin (53.11%), and erythromycin (23.44%) antibiotics, respectively.

#### CONCLUSION

To the best of our knowledge, this is the first study regarding the antimicrobial susceptibility of *C. perfringens* in small ruminants in Kerman province of Iran. This pattern of antibiotic resistance in *C. perfringens*, potentially reflecting the farm usage of these agents. Tight restriction of unnecessary antibiotic uses is necessary for some clostridial diseases.

**Keywords:** *Clostridium perfringens*; Macrolide; Tylosin; Erythromycin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# High prevalence of $\beta$ -lactam and aminoglycoside resistance in *Clostridium perfringens* isolates from food producing-animals in Kerman, Iran

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Clostridium perfringens* (*C. perfringens*) is a ubiquitous, anaerobic, Gram-positive and spore-forming bacteria, ranking amongst the most important pathogens living in the intestinal tracts of both humans and animals. Under certain conditions, *C. perfringens* may become an opportunistic pathogen and over-proliferate, producing a wide spectrum of toxins that can lead to its pathogenic effects. Meanwhile, *C. perfringens* is recognized as one of the most important foodborne pathogens in humans; and considered as one of the leading cause of nosocomial diarrhea. So, emergence of antibiotic-resistant strains makes the dealing with the bacteria more challenging. The objective of this study was to determine the resistance of *C. perfringens* isolates to  $\beta$ -lactam and aminoglycoside antibiotic classes.

### MATERIALS AND METHODS

A total of 273 *C. perfringens* isolates, which were previously recovered from fecal samples of the diseased goats and sheep from Kerman province in southeast of Iran, evaluated for antimicrobial resistancy against the penicillin ( $\beta$ -lactam) and streptomycin (aminoglycoside) antibiotics by Kirby-Bauer disk diffusion method.

### RESULTS AND DISCUSSION

The findings of our study demonstrated the high antibiotic resistant rate of *C. perfringens* isolates in food producing-animals. The highest resistancy were observed to streptomycin (83.51%) and then penicillin (41.02%) antibiotics, respectively.

### CONCLUSION

To the best of our knowledge, this is the first study regarding the antimicrobial susceptibility of *C. perfringens* in food-animals in Kerman province of Iran. The present findings provide supplementary substantiation for the potential involvement of animals as a reservoir for the resistant *C. perfringens* strains.

**Keywords:** *Clostridium perfringens*;  $\beta$ -lactam; Aminoglycoside; Penicillin; Streptomycin

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Comparative Assessment of ELISA and MLD for Measurement of Epsilon Toxin in Enterotoxemia Vaccine Production Process

Maryam Amini, Mehrdad Shamsaddini Bafti, Majid Ezatkah, Fatemeh Hekmatinia, Alireza Karimabadi

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#### ABSTRACT

One of the most common veterinary biologicals is enterotoxemia vaccines that produced against *Clostridium perfringens*. Enterotoxemia diseases are mediated by toxins and epsilon is the most pathogenic toxin so killed vaccines comprise toxoided forms of these toxins. During vaccine manufacture toxin production is measured by Minimum Lethal Dose (MLD) tests that rely on animal testing in mice that measures the minimum concentration of toxins.

The objective of the present study was to compare the performance of ELISA assay that quantify epsilon toxins and toxoids with MLD and correlation evaluation. Suitable *in vitro* assays would completely replace the use of animals in testing at these stages, furthermore ELISA test has the ability to detect toxin and toxoids while MLD detect the active toxins only.

20 supernatant sample of type D fermenter culture which contain active toxin used in this research for MLD test after activation with trypsin serial dilutions was done and for each dilution, 0.5 ml was inoculated intravenously into each of two mice. For ELISA assay microtitration plates coated with proper dilutions of supernatant samples and standard toxins as positive control and other stages was performed and results interpreted. Pearson correlation coefficient and linear regression analysis used for the correlation between MLD and ELISA results.

Correlation coefficient greater than zero indicates a positive relationship between two tests so precise replacements of ELISA by mouse tests in this stage would significantly reduce animal usage in vaccine production and is a suitable replacement for very painful, stressful and prone to high variability assay.

**Keywords:** *Clostridium perfringens*, Epsilon toxin, MLD, ELISA

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Distribution of minor virulence genes among *Clostridium perfringens* Isolates

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#### ABSTRACT

*Clostridium perfringens* (*C. perfringens*) belongs to the family of *Clostridiaceae* and produces a wide range of toxins (four major and a variety of minor toxins). Some toxins associated with virulence have been shown to participate in the pathogenesis of enteric diseases in sheep, goats, and other animals. The aim of this study was to determine the presence of some minor virulence genes and their genetic diversity in *C. perfringens* isolates.

About 84 isolates collected from sheep and goat flocks were provided by the microbial archive of Razi Institute (south-east branch) that located in Kerman. Isolates were removed from the cryotube stored at -70° C; then, it smeared on blood agar medium containing 5 % defibrinated sheep blood, which was incubated in an anaerobic condition. After DNA extraction, detection of toxin genes (*cpb2*, *tpel*, and *PFO*) was carried out, using three pairs of specific primers were examined and sequenced for the presence of minor virulence genes (*PFO*, *cpb2* and *tpel*) by PCR method. Purified PCR products were sequenced using the Sinacolon facility, Tehran, Iran. The gene sequences were aligned according to their nucleotides, using computer program MEGA 7. Then, analysis was performed for confirm the nucleotide sequences.

Results showed that *PFO* and *cpb2* were found in 79 out of 84 (94.4 %) and the presence of *tpel* was confirmed in 29 out of 84 (35 %) isolates so the dominant minor virulence genes were *PFO* and *cpb2*. prevalence of these genes in *C. perfringens* isolates would provide more information regarding the importance of these toxins and lead to a greater understanding of the pathogenesis of diseases caused by *C. perfringens*, furthermore DNA sequencing revealed closed relationships with others world strains that were range approximately (97–100 %) with the GenBank database.

**Keywords:** *C. perfringens*, minor virulence genes, Sequence, PCR

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Circulating *Brucella* spp. isolates in dairy cattle farms of Iran

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

Brucellosis is a widespread disease in developing countries like Iran. Cattle are mainly affected by *Brucella abortus*, although *Brucella melitensis* and *Brucella suis* can also sometimes cause infections in cattle. Common symptoms in female cattle include abortion and the retention of the fetal membrane, while males may exhibit signs of orchitis and bursitis. The transmission of brucellosis typically occurs through direct or indirect contact with infected cattle or their bodily fluids. This study aimed to determine the prevalence of brucellosis in cattle slaughtered in the Iranian slaughterhouses and identify the *Brucella* species circulating among these animals

#### MATERIALS AND METHODS

. A total of 525 cattle lymph node samples during 2021 and 2022 were collected from the different provinces of Iran and analysed by culture. Following the culture assay, all isolated bacteria were subjected to phage typing and AMOS PCR analysis.

#### RESULTS AND DISCUSSION

The prevalence of brucellosis in different locations was 19.4%, 12.4%, 0.9% and 0.3% for *Brucella melitensis* biovar 1, *Brucella abortus* biovar 3, *Brucella melitensis* biovar 3 and RB51 vaccine respectively. The 174 isolated samples were confirmed with PCR.

#### CONCLUSION

Implementing effective management techniques and raising public awareness about the transmission of brucellosis are crucial. Furthermore, there is a need for additional research on brucellosis in high-risk farms.

**Keywords:** *Brucella melitensis*, *Brucella abortus*, *Brucella suis*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Investigating the effect of silver nanoparticles on biodeteriogen fungi isolated from Masjed-e Jāmé of Isfahan

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

Biodeterioration is an irreversible damage caused by microorganisms or their metabolites to artworks and monuments. Fungi are one of the most important agents in the biodeterioration of cultural works in external environments. Nowadays, nanoparticles obtained from plant extracts have been attracted the attention of researchers to control and treat the biodeterioration due to their availability, effectiveness, low cost and compatibility with the environment. The aim of this study is to investigate the antifungal properties of silver nanoparticles prepared from the aqueous extract of walnut (*Juglans regia*) skin against 6 deteriogen fungi isolated from the plaster, brick and ceramic surfaces of Isfahan Jame Mosque.

### MATERIALS AND METHODS

In this study, the antifungal effect of silver nanoparticle on the selected fungi with deteriorative effect was performed using disk diffusion method and microtiter plate. Then, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) was determined.

### RESULTS AND DISCUSSION

Silver nanoparticle at  $2 \times 10^4$   $\mu\text{g/ml}$  concentration had the greatest effect on *Parengyodontium album* with inhibition zone 24 mm, and MIC 625  $\mu\text{g/ml}$ . Furthermore, the lowest effect of silver nanoparticle was on *Aspergillus flavus* with inhibition zone 12 mm and MIC of  $2 \times 10^3$   $\mu\text{g/ml}$  without any fungicidal effect.

### CONCLUSION

According to the results of this study, it is concluded that silver nanoparticles are effective on fungi, which are biodeteriogen in cultural heritage buildings, although more investigation should be done for the efficiency of this nanoparticle on stone.

**Keywords:** Antifungals, Biodeterioration, Biological nanoparticle, Cultural heritage, Green synthesis

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### *Deinococcus Radiodurant* Bacteria

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

*Deinococcus radiodurans* is a radiation-resistant species that can survive in radioactive environments where other organisms cannot grow. Numerous studies speculate that several antioxidant and DNA repairing proteins are involved in its partial resistance to radiation. PPRI protein is an essential regulatory protein playing roles in *Deinococcus radiodurans's* radiation resistance. These bacteria are known to be the most resistant bacteria against high gamma radiation, ultraviolet arrays, chemical mutagens, or long-term drying. It is also found in certain species that tolerate high temperatures, toxic metals or organic solvents, and powerful enzymes. Gamma radiation have always been known as a lethal factor for living cells, as they damage the DNA and cause cell death. But *Deinococcus radiodurans* can tolerate very high doses of gamma radiation, approximately 3,000 times the dose that causes harm to humans. This review summary focuses on special applications of *Deinococcus radiodurans* in a wide range of biotechnological fields.

The versatile bacterium *Deinococcus radiodurans* offers a range of applications across healthcare, environmental remediation, industry, and space exploration. In healthcare, its crude secondary metabolite extract (CSME) displays antiproliferative effects, selectively inducing apoptosis in cancer cells without harming normal ones. Key antioxidants, deinoxanthin and DeinoPol, hold promise in cosmetics and pharmaceuticals for their ROS-absorbing capabilities and radiation protection. In radioactive waste cleanup, *D. radiodurans's* resistance to radiation makes it a strong candidate, with genetic engineering strategies enhancing its bioremediation capabilities, even tackling uranium precipitation and capturing radioactive iodine. In industry, *Deinococcus* enzymes like amylosucrase (DgAS) offer stability and versatility, used in cosmetics, surfactant production, and food applications. Moreover, *D. radiodurans* proves invaluable in space exploration due to its resilience to radiation and desiccation, enabling repair of DNA even in microgravity. Enhanced resistance mechanisms, like PPRI protein expression, further bolster its relevance in space missions. Altogether, *D. radiodurans* presents a wealth of opportunities across various fields, from healthcare to space travel.

#### CONCLUSION

*Deinococcus radiodurans* is a remarkable bacterium that can survive and grow in extreme conditions that are lethal for most living organisms. It has a unique ability to repair its damaged DNA and proteins in a rapid and efficient manner. It also possesses several antioxidant enzymes and molecules that protect it from oxidative stress. The PPRI protein is one of the key regulators of its radiation resistance. *D. radiodurans* has potential applications in various fields, such as bioremediation, bioengineering, astrobiology, and medicine. It is a fascinating model organism for studying the molecular mechanisms of stress resistance and adaptation.

**Keywords: Extremophile, Radiophile, Thermophile, Radiotherapy**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Induction of Immunity by PilS<sub>2</sub> Recombinant Protein Against *Pseudomonas Aeruginosa* Infections: A Mouse Study

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## ABSTRACT

### BACKGROUND AND OBJECTIVE

*Pseudomonas aeruginosa*, a gram-negative opportunistic bacterium, is a leading cause of nosocomial infections and a major contributor to mortality despite advances in management. Among virulence factors, subtype b of Type IV pili (PilS<sub>2</sub>) has emerged as a potential target for preventive and therapeutic investigations. However, effective immunity induction against *Pseudomonas aeruginosa* remains a challenge.

### MATERIALS AND METHODS

In this study, we evaluated the induction of immunity in BALB/c mice by administering PilS<sub>2</sub> recombinant protein. Four groups of mice were injected with PBS, Alum adjuvant, PilS<sub>2</sub> recombinant protein alone, or PilS<sub>2</sub> recombinant protein with Alum adjuvant on days 0, 14, and 28. After two weeks from the third injection, we assessed the levels of total IgG, IgG1, and IgG2b using indirect ELISA.

### RESULTS AND DISCUSSION

The results revealed that the PilS<sub>2</sub> and PilS<sub>2</sub> + Alum groups exhibited significantly higher levels of total IgG antibodies than the control groups ( $p < 0.001$ ). Moreover, the specific IgG1 levels in mice injected with PilS<sub>2</sub> alone and PilS<sub>2</sub> + Alum were significantly elevated compared to the control group ( $P < 0.05$ ). Similarly, the specific IgG2b antibody titres in the PilS<sub>2</sub> and PilS<sub>2</sub> + Alum groups were significantly higher than those in the control groups ( $p < 0.05$ ). assessment of induced immunity in BALB/c mice that received PilS<sub>2</sub> recombinant protein in *Pseudomonas aeruginosa*.

### CONCLUSION

This study demonstrates that the administration of PilS<sub>2</sub> recombinant protein effectively induces humoral immune responses against *Pseudomonas aeruginosa*. The inclusion of Alum adjuvant further enhances the immune response, as evidenced by the increased levels of total IgG, IgG1, and IgG2b antibodies. These findings provide valuable insights into the potential of PilS<sub>2</sub> as an immunogen for the development of preventive and therapeutic strategies against *Pseudomonas aeruginosa* infections.

**Keywords:** *Pseudomonas aeruginosa*, PilS<sub>2</sub>, Type IV pili.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Study on pathogenic role of *Demodex* mites in Several diseases

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVE

The skin is the human body's largest organ, colonized by a diverse milieu of microorganisms, most of which are harmless or even beneficial to their host. *Demodex folliculorum* and *Demodex brevis* are two species of tiny parasitic mites that live in the hair follicles and sebaceous glands of human skin, respectively. *Demodex* has been considered to be related with multiple skin disorders, but controversy persists. Demodex mites play an important role in the occurrence of diseases such as seborrheic dermatitis, rosacea, acne vulgaris, itching and hair loss.

#### MATERIALS AND METHODS

In this case-control study, the prevalence of Demodex mites was studied in facial biopsy of 100 patients with rosacea, 90 patients with seborrheic dermatitis, 85 patients with acne, 100 patients with hair loss and 65 patients with itching. Direct microscopic examination was done from 1 cm<sup>2</sup> of facial skin and scalp sebum. Some references consider the density of more than five mites per cm<sup>2</sup> as a pathogenic criterion.

#### RESULTS AND DISCUSSION

We have separately investigated the relationship between *Demodex* mites and skin diseases such as rosacea, seborrheic dermatitis, hair loss, itching and acne. The prevalence of Demodex mites in patients with rosacea, seborrheic dermatitis, acne, hair loss and itching were 84%, 72%, 52% and 60%, respectively. Complications had significantly higher prevalence and degrees of Demodex mites contamination compared to control patients.

#### CONCLUSION

Demodex mites live inside almost every human's hair follicles. The mites usually don't cause any problems, but if they multiply too much, they can cause demodicosis. Developing *Demodex* mites may be causative agents of occurrence skin diseases through various mechanisms: They may mechanically block hair follicles, secrete digestive enzymes, destroy the epithelial barrier, trigger reactions of the immune system. According to our study, the prevalence of Demodex mites in patients with rosacea (84%) was significantly higher than the patients with seborrheic dermatitis, acne, hair loss, itching. So Demodex mites can play an important role in the etiology of these diseases especially rosacea.

**Keywords:** *Demodex*, diseases of *Demodex*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Identification of the role of *H. pylori* in HNSCC tumors and margin healthy tissues and its association with demographic and clinicopathologic characteristics

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Helicobacter pylori* (*H. pylori*) is a bacterium known for its association with gastric disease, but its presence and role in extra-gastric sites, such as head and neck squamous cell carcinoma (HNSCC), have gained attention. The aim of this study was to investigate the detection of *H. pylori* in HNSCC tumors and margin healthy tissues and its possible association with demographic and clinicopathologic features.

### MATERIALS AND METHODS

A total of 62 patients diagnosed with HNSCC were enrolled in this study. Tumor and margin healthy tissues were obtained during surgery and *Helicobacter pylori* was diagnosed by PCR method targeting *glmM* and *16S rRNA* genes. Demographic information, including age and sex, and clinicopathologic characteristics such as tumor stage, lymph node involvement, and histological grade were obtained from medical records.

### RESULTS AND DISCUSSION

In 62 HNSCC patients, *Helicobacter pylori* was detected in 32.25% of tumor tissues and 11.29% of margin healthy tissues. Statistical analysis showed that there was a significant association between *Helicobacter pylori* infection in tumor tissue and margin healthy tissues ( $P = 0.001$ ). Although most cases with positive *H. pylori* infection in tumor tissue were at advanced stages compared with early stages (60%), the presence of *H. pylori* in tumor tissue showed no statistically significant association with the stage of tumor progression ( $P=0.05$ ). In addition, there was no significant association between the detection of *H. pylori* in tumor tissues and gender, age, alcohol, smoking, opium consumption, and histological grade. Also, in healthy peripheral tissues, *H. pylori* diagnosis showed no significant association with demographic or clinicopathological characteristics.

### CONCLUSION

This study demonstrated the presence of *H. pylori* in HNSCC tumor tissues and margin-healthy tissues. Detection of *Helicobacter pylori* in most tumor tissues was associated with advanced tumor stage. Although 65% of *H. pylori*-positive cases were over 61 years of age, this association was not significant. These results suggest a potential role of *H. pylori* in HNSCC development and progression. Further research is needed to elucidate the underlying mechanisms and explore potential therapeutic implications.

**Keywords:** Grade, head and neck squamous cell carcinoma, *Helicobacter pylori*

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The Antiviral Potential of Medicinal Plants in Treating Viral Infections

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### ABSTRACT

Recently, there has been remarkable progress in the field of antiviral herbal therapy owing to increasing concerns about the development of drug resistance and limited advances in the field of antiviral drug discovery. In almost all countries, medicinal plants have been widely used throughout history for the treatment of diseases and infections as traditional healing remedies due to their broad therapeutic spectrum and minimal or no side effects. As synthetic antiviral drugs are not available against most of the viral agents, hence all possible efforts have been focused on the search for new drugs and complementary alternative medicines from different herbal formulations. Despite their long history of use, the research and scientific evidence regarding the use of medicinal plants and natural products as prophylactics, therapeutics, and their health multiple beneficial applications have only gained momentum in the past few decades. Many scientific studies have been undertaken, which range from the separation of active substances to the comprehension of the therapeutic mechanisms of antiviral herbs, their potent applications in the neutralization of viral pathogens and clinical trials. Consequently, hundreds of herbs and plant metabolites have been screened, identified, and tested for their antiviral activities; fortunately, some have shown significant medicinal activity in the preventing or ameliorating of various viral diseases such as: Rabies, Influenza types A, B and C, Hepatitis B and C in both preclinical and clinical studies.

**Keywords:** Influenza virus, Antiviral, Natural product, Medicinal plant

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Apoptotic potential of biogenically synthesized selenium nanoparticles with *Lactobacillus casei* supernatant against breast cancer cells

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

Nowadays, cancer is the most common life-threatening disease which is spreading due to lifestyle. The use of nanoparticles in biomedical research has been increasingly developed in recent years. The aim of this study was to investigate the anticancer effects of biogenic synthesized selenium nanoparticles (SeNPs) using cell-free supernatant of *Lactobacillus casei* on MCF-7 cancer cell line.

### MATERIALS AND METHODS

The prepared SeNPs were characterized by XRD, Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), and energy dispersive spectroscopy (EDX), and Zeta potential.

### RESULTS AND DISCUSSION

The SEM images demonstrated the spherical morphology of the synthesized nanoparticles. Treatment of breast cancer cells with different concentrations of nanoparticles showed that the nanoparticles had the ability to inhibit MCF-7 proliferation, and flow cytometry showed that SeNPs could induce apoptosis in MCF-7 cells. Gene expression analysis showed that after treatment of MCF-7 cells with SeNPs, the expression levels of *CASP3*, *CASP9* and *BAX* genes were increased. SeNPs could also prevent migration and invasion of MCF-7 cancer cells.

### CONCLUSION

These finding indicated that SeNPs induced G1 phase arrest and apoptosis in MCF-7 cells. The process used in this study to develop the nanoparticles is novel and should have useful anticancer applications in the future, mainly because of proper specific targeting to cancer cells. SeNPs may serve as a potential therapeutic agent for breast cancer.

**Keywords:** Apoptosis, Breast Cancer, *Lactobacillus casei*, Selenium Nanoparticles, Cell cycle arrest

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The role of gut microbiome in animal health and disease

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

The digestive system is crucial for animal health, converting food into nutrients for energy, development and tissue repair. Obtaining methods to increase and improve the function of the digestive system, optimizes productivity, growth, reproduction and milk production.

#### MATERIALS AND METHODS

Data about the role of gut microbiome in animal health and disease were gathered using scientific search engines including Web of Science, Wiley Online, Science Direct, Scopus, and Google Scholar.

#### RESULTS AND DISCUSSION

The gut microbiota has a critical role in regulating metabolism, sustaining the immune system and avoiding disease in animals. It is essential for digestion, absorption and energy metabolism in dogs, cats and agricultural animals. According to recent studies, the health of farm animals may also be evaluated using the gut microbiome, Dysbiosis might be treated by employing techniques such as faecal microbiota transplantation (FMT).

So, dysbiosis can be caused by a variety of reasons, such as: antibiotic therapy, stress and eating environmental contaminants. Unbalances in the gut microbiome have been related to a number of disease in animals, such as gastrointestinal (GI) disorders, obesity, metabolic disorders, immunological disorders inflammatory bowel disease (IBD) and neurobehavioral disorders.

Use of probiotics and prebiotics can help animals' gut microbiomes. The health of the host animal is improved by probiotics such as *Acidophilus Lactobacillus*, *Faecal Enterococci* and *Bacillus* species, which enhance the microbial balance in the gut. Prebiotics that support the growth and activity of beneficial bacteria in the gut include: Fructooligosaccharides (FOS), Galactooligosaccharides (GOS) and Mannan oligosaccharides (MOS).

#### CONCLUSION

So, maintaining the balance of the microbiome plays a significant role in the health of the digestive system in animals.

**Keywords: Microbiome, Disorders, Animal health**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# Immunoinformatics aided design of a new epitope-based vaccine candidate targeting pneumococcal histidine triad protein D (PhtD)

**Mona Shafaghi<sup>1,2,3</sup>, Zohreh Bahadori<sup>1,2,3</sup>, Hamid Madanchi<sup>1,2,4</sup>, Mohammad Mehdi Ranjbar<sup>5</sup>, Ali Akbar Shabani<sup>1,2,\*</sup>, Seyed Fazlollah Mousavi<sup>3,\*</sup>**

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## ABSTRACT

### BACKGROUND AND OBJECTIVES

*Streptococcus pneumoniae* (pneumococcus) is a major respiratory pathogen that causes high levels of mortality and morbidity in infants and the elderly. Despite the use of antibiotics and vaccines, fatal pneumococcal disease remains prevalent. Due to drawbacks of the current polysaccharide-based vaccine, the most promising way to generate an improved vaccine may be to utilize pneumococcal protein antigens with different roles in virulence. Pneumococcal histidine triad protein D (PhtD), a highly immunogenic surface protein produced by all strains of *S. pneumoniae*, can elicit protective immunity against fatal pneumococcal infections.

### MATERIALS AND METHODS

In this study, immunoinformatics approach was used to design an effective epitope-based vaccine against pneumococcal strains based on PhtD protein. The high-scored B- and T-cell epitopes overlapping with each other shared between different servers were considered, and the final peptides from PhtD protein were linked together with suitable linker. The prediction of the physicochemical and immunological properties, antigenicity, allergenicity, toxicity, 3D model, and structural B cell epitopes in the final construct, as well as molecular docking of the final model with HLA receptor and immune simulation were carried out using computational tools.

### RESULTS AND DISCUSSION

The evaluation of the characteristics showed that the final construct is stable, soluble, antigenic and non-allergenic. In addition, it was found that the protein has an acidic and hydrophilic nature. The 3D model was constructed and refined, and the Ramachandran plot, ProSA-web and ERRAT confirmed the quality of the final model. Molecular docking analysis showed that the developed construct could strongly interact with HLA receptor. Finally, codon adaptation was performed for gene expression in *E. coli* followed by *in silico* cloning in the pET28a(+) plasmid. Computational analysis revealed that the designed construct passed the evaluations with satisfactory scores and had the potential to induce robust immune responses.

### CONCLUSION

For the first time, this study presents a new vaccine containing the dominant epitopic regions of the PhtD antigen. *In silico* analysis showed acceptable results, however, the proposed vaccine candidate should be experimentally verified in the laboratory to ensure its safety and immunogenicity.

**Keywords:** *Streptococcus pneumoniae*, Pneumococcal histidine triad protein D (PhtD), computational tools, Immunoinformatics, epitope-based vaccine candidate.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### *In silico* design of a novel multi-peptide vaccine candidate against pneumococcal surface protein A (PspA) families 1 and 2

**Mona Shafaghi<sup>1,2,3</sup>, Zohreh Bahadori<sup>1,2,3</sup>, Hamid Madanchi<sup>1,2,4</sup>, Mohammad Mehdi Ranjbar<sup>5</sup>, Ali Akbar Shabani<sup>1,2,\*</sup>, Seyed Fazlollah Mousavi<sup>3,\*</sup>**

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#### ABSTRACT

#### BACKGROUND AND OBJECTIVES

*Streptococcus pneumoniae* has remained a leading cause of fatal infections such as pneumonia, meningitis and sepsis. High morbidity and mortality are the main concern for pneumococcal vaccine development. Due to the limitations of currently marketed polysaccharide-based vaccines, non-serotype-specific protein-based vaccines have received wide research interest in recent years. One step further is to identify high antigenic regions within highly-conserved proteins in order to develop peptide vaccines, providing broader serotype coverage and more effective protection. Pneumococcal surface protein A (PspA), a highly immunogenic surface protein produced by almost all pneumococcal strains, can elicit protective immunity against fatal pneumococcal infection.

#### MATERIALS AND METHODS

In this study, immunoinformatics tools were used to design an effective multi-peptide vaccine based on PspA protein (families 1 and 2) against multiple strains of pneumococcus. The high-scoring B- and T-cell epitopes overlapping with each other shared between different servers were considered, and the final peptides from PspA families 1&2 were selected and merged together with suitable linkers. The prediction of physicochemical properties, antigenicity, allergenicity, toxicity, 3D-structure, and conformational B cell epitopes in final construct, as well as molecular docking of final construct with HLA receptor and immune simulation were carried out using computational tools.

#### RESULTS AND DISCUSSION

The evaluation of the properties showed that final construct was soluble, antigenic, and non-allergenic. Furthermore, the protein was found to be acidic and hydrophilic in nature. The 3D-structure was built and refined, and the Ramachandran plot, ProSA-web, and ERRAT validated the quality of final model. Molecular docking analysis showed that the designed construct could interact strongly with HLA. Finally, codon optimization was performed for gene expression in *E. coli*, followed by *in silico* cloning in the pET28a(+) vector. The computational analysis showed that the final construct passed the evaluations with satisfactory scores and had potential to elicit robust immune responses.

#### CONCLUSION

For the first time this study presents a novel vaccine containing of the immunodominant regions of PspA antigens. The *in silico* analysis revealed acceptable results, however, the suggested vaccine needs to be experimentally confirmed in laboratory to ensure its safety and immunogenicity.

**Keywords:** *Streptococcus pneumoniae*, Pneumococcal surface protein A (PspA), Immunoinformatics, computational tools, Multi-peptide vaccine.

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

# The role of intestinal microbiota and mucosal barrier in the pathogenicity of *E. histolytica*: a systematic review

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## ABSTRACT

### BACKGROUND AND ABJECTIVE

*E. histolytica* is a protozoan that causes intestinal and extra-intestinal amoebiasis. Changes in the intestinal microbiota can lead to symptomatic progression in previously asymptomatic patients. Dysbiosis in the microbiota, caused by factors like antibiotic use and immunodeficiency, weakens the innate defense against *E. histolytica*. Probiotic bacteria have the potential to prevent *E. histolytica* from reaching the intestinal epithelium and reduce its pathogenicity. *Prevotella copri*, a bacterium in the intestinal flora, plays a role in the pathogenicity of *E. histolytica* and is depleted in infected individuals.

### MATERIALS AND METHODS

This systematic review followed PRISMA guidelines and focused on studies from 2010 to 2020. The search was conducted on databases including Scopus, PubMed, Science Direct, Google Scholar, and Web of Science. After applying inclusion and exclusion criteria, a total of 73 articles were identified. Two researchers independently performed data extraction and quality assessment, with a third researcher providing consensus. The selected articles were analyzed for relevant information on the association between gut microbiota and *E. histolytica*.

### RESULTS AND DISCUSSION

Studies conducted between 2010 and 2020 revealed changes in the intestinal microbiota of individuals infected with *E. histolytica*. These changes involve an increase in bacterial families such as Clostridium, Coccoid, Fusobacter, and Bacteroids, while the populations of Bifidobacterium and Ruminococcaceae decrease. Additionally, significant alterations occur in the anaerobic bacterial population, including a decrease in *Prevotella copri*. These findings highlight the potential impact of intestinal microbiota on the pathogenicity of *E. histolytica*.

### CONCLUSION

Dysbiosis in the intestinal microbiota facilitates the pathogenesis of *E. histolytica* and impairs the immune response. Imbalances in the normal intestinal flora can cause asymptomatic individuals to progress to symptomatic disease. Future investigations are needed to explore the potential use of probiotics as biological interventions for managing *E. histolytica*-related complications and whether *E. histolytica* disrupts the normal intestinal flora.

**Keywords:** *Entamoeba Histolytica*, *E. Histolytica*, Gut Microbiota

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### Common Treatment of Demodex Mites

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#### ABSTRACT

#### BACKGROUND AND ABJECTIVE

Demodex is a microscopic mite that naturally resides on the skin of humans and animals. they live in hair follicles or sebaceous glands. an overgrowth or infestation can lead to various skin conditions. Demodex can cause diseases such as blepharitis, rosacea, seborrheic dermatitis, hair loss and acne. With diagnostic methods, if the density is more than 5 Demodex in 1 square centimeter, the practitioner will start the treatment for the patient. Topical medications are often the first line of treatment for Demodex infestations. Prescription creams, gels, and lotions or ointments are commonly used to kill Demodex mites and reduce associated symptoms. In more severe cases or when topical treatments are ineffective, oral medications may be prescribed to control Demodex populations.

#### MATERIALS AND METHODS

after studying many articles with key words: Demodex mites, treatments, rosacea, mites, Demodex diseases, from data bases such as pubmed, researchgate, google scholar and etc, data have collected.

#### RESULTS AND DISCUSSION

Prescription creams, gels, or ointments containing active ingredients such as metronidazole, permethrin, sulfur, or tea tree oil are commonly used to kill the mites and reduce associated symptoms. In more severe cases or when topical treatments are ineffective, prescribed oral Ivermectin, an anti-parasitic medication, is frequently used to treat Demodex infestations. It works by interfering with the mites' nervous system, ultimately leading to their death.

#### CONCLUSION

It is recommended to avoid the use of oral and chemical drugs as much as possible due to the high complications and negative effects they may have on other organs of the body. The best and safest treatment is the use of face wash solutions and gels, shampoos and topical creams that contain tea tree oil (Specific percentage). Due to being less dangerous and being herbal, they are more useful for destroying demodex mites. Maintaining good hygiene practices is crucial in managing Demodex infestations. Regularly washing the affected areas with mild cleansers (with tea tree oil) can help keep the mite population under control. Treating underlying conditions can help improve symptoms associated with Demodex. Dermatologists may prescribe specific medications or recommend skincare routines tailored to address both the primary skin condition and the Demodex infestation.

**Keywords: Demodex Mites, Tea Tree Oil, Ivermectin, Permethrin and Treatment**

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## 24<sup>th</sup> IRAN'S INTERNATIONAL CONGRESS OF MICROBIOLOGY

### The role of gut microbiota in Parkinson's disease

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#### ABSTRACT

##### BACKGROUND AND OBJECTIVES

The gut microbiota, which is now recognized as an important organ of the human body, can have an impact on various neurological outcomes such as cognition, learning, and memory. In addition, the gut microbiota can regulate brain growth and behavior, which plays a role in several neurological disorders such as Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, anxiety, stress, and others.

##### MATERIALS AND METHODS

In this study, we aim to investigate the relationship between gut microbiota and Parkinson's disease and to highlight appropriate microbiota-based therapeutic approaches for Parkinson's disease. The approaches examined in this study provide a foundation for identifying the mechanisms of the gut microbiome's ability to influence the brain and host behavior. Additionally, a deeper understanding of the gut-brain axis may establish a connection between gut microbiota and the pathogenesis of Parkinson's disease and may aid in the prevention or early diagnosis of Parkinson's disease.

##### RESULTS AND DISCUSSION

Fecal microbiota transplant, prebiotic, and probiotic laid the groundwork for identifying the mechanisms underlying the ability of the gut microbiota to influence host's brain and behavior. It could help in preventing or early diagnosis of Parkinson's disease possibly through some peripheral biomarkers. If this hypothesis is valid and that gut microbiota is involved in Parkinson's disease etiology, we might be looking at a new therapeutic and treatment regimen probably focused on dietary and pharmacological interventions to maintain healthy gut microbiota.

##### CONCLUSION

Recent studies have also shown that patients with Parkinson's disease have a disruption in gut microbiota balance (dysbiosis), but the exact role of this in causing the neurological disorder is still unknown. However, new approaches based on gut microbiota can be developed for the prevention and treatment of neurological disorders such as Alzheimer's.

**Keywords:** Microbiome, Gut-brain axis, Parkinson's disease, Probiotics, Fecal Microbiota Transplantation

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