UMS 24

SERIES

4th - 6th April 2024

MONA PLAZA HOTEL, **Belgrade, Serbia**

XIII CONGRESS OF MICROBIOLOGISTS OF SERBIA

with international participation

MIKROMED REGIO 5

FROM BIOTECHNOLOGY TO HUMAN AND PLANETARY HEALTH

BOOK OF ABSTRACTS

ORGANIZER:

SERBIAN SOCIETY FOR MICROBIOLOGY

SUPPORTED BY:

Federation of European Microbiological Societies

Republic of Serbia

MINISTRY OF SCIENCE, TECHNOLOGICAL DEVELOPMENT AND INNOVATION

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President of the Serbian Society for Microbiology

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Content

Message from the organizing commitee

Dear colleagues and friends,

The objective of the event was to present the latest developments in microbiology that contributed to a better understanding of the role of microorganisms in nature and to bring together microbiologists from Serbia and the region with professionals from all over Europe, including microbiologists of various disciplines, bioinformaticians, geneticists, molecular biologists, biochemists, epidemiologists, pediatricians, infectious disease physicians, and all other scientists with common interests.

This regional meeting addressed all prevailing microbiological issues and offered solutions to overcome them by world-class experts in the field. The resistance of microorganisms to antimicrobial drugs is causing major problems in veterinary and human medicine, necessitating the improvement of vaccines and the discovery of new drugs, but also alternative treatment models. Growing antimicrobial resistance, especially biofilm-related, requires alternative measures to biocontrol the spread of the microorganisms in various environments. These sessions discussed possible alternatives to common antimicrobials, ranging from bacteriophage applications, new natural compounds biotechnology or nanotechnology, as well as biological control for the inactivation of pathogenic and/or resistant phenotypes of microorganisms.

In addition, food manufacturers and retailers have been trying for decades to reduce the material damage and risks to human health posed by biofilms in food processing facilities. The environment is already too polluted by many human missteps, so any help from microorganisms to remove or process waste materials can be a big help. We are getting better and better at using microorganisms in technological processes, firstly in the medical field, but also in agriculture, industry and the energy sectors.

Our knowledge of how microbial diversity is distributed in natural environments and how microbes influence ecosystems is constantly evolving as public databases are established and new techniques based on massive sequencing are developed. The microbiomes found in anthropogenic environments and on human-made materials are generally much less complex than those found in natural environments. Despite this simplicity, it is very difficult to link cause and effect when it comes to determining the role of individual microorganisms. Improved genome engineering tools in model organisms allow for a comprehensive remodeling of metabolic and regulatory networks.

At the same time, a growing number of non-model organisms can be modified with different traits so that they can be further used in different applications and environments. This expanded range of engineering capabilities and modified species brings their application in the real world closer and has the potential to make a real contribution to sustainability and addressing global health challenges.

Microorganisms are the key drivers of ecosystem functions, and microbial diversity plays a central role in maintaining the stability and sustainability of ecosystems. These sessions were examined some of the principles that shape and maintain this biodiversity and explore the factors that shape microbiomes and contribute to the success of specific members of communities in different habitats.

Presentations were focused on omics techniques such as metagenomics, metatranscriptomics, proteomics and metabolomics, which are used to better understand why the health of humans, animals and plants depends on microbial interactions. In this way, the complex microbiomes and the interactions between the microbiota and a variety of host organisms from different domains of life were explored.

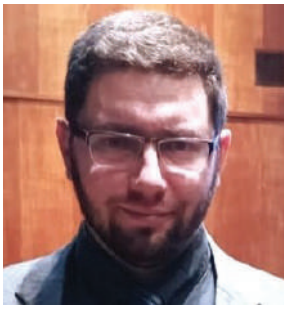
We strongly believe that the Congress was an excellent place to exchange and combine scientific ideas among experts and participants, with great opportunities to start new international collaborations and joint scientific projects. We have received an overwhelming response to our call, with numerous talented applicants, more than 350 participants from more than 20 countries (Austria, Belgium, Bosnia and Herzegovina, Croatia, France, Georgia, Germany, Greece, Hungary, India, Iraq, Italy, Montenegro, Namibia, North Macedonia, Portugal, Russian Federation, Serbia, Slovenia, Netherlands and United States) applying for the limited number of available grant awards (we have accepted 29 participants). In addition to presentations by invited speakers, the programme also included poster presentations by young researchers and PhD students. We were honoured to welcome 62 lecturers, 15 offered talks and 8 panelists and presenting cases. We have organized oral presentations in 15 paralel sessions, complemented by two panel discussions and a workshop entitled "NGS in Microbiology". We would like to thank all participants for their scientific commitment, especially for the more than 170 abstracts submitted, which contributed significantly to the success of the Congress. The Congress is accre ditated by the Health Council of the Republic of Serbia under the registration number A-1-185/24.

We hope you enjoyed the Congress programme and found it stimulating and informative. We also hope that you enjoyed the beauty of Belgrade and the Serbian hospitality. We sincerely wish you health, love and happiness and look forward to the new meetings.

Sincerely,

Ivica Dimkić
University of Belgrade
Faculty of Biology, Serbia

Scientific
Committee
Chairperson



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Faculty of Medicine, Serbia

Organizing
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Lazar Ranin
President of the Serbian Society
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General information

CONGRESS VENUE

The meeting was held at the Hall „Donji Dorćol“ and Hall „SCHONDA 4“, Mona PLAZA hotel, located at Cara Uroša 62-64, Belgrade, Serbia; Hall „Beogradska panorama“ and Hall „Club“, situated Hotel Palace, Topličin Venac 23, Belgrade, Serbia and Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 444a, Belgrade, Serbia.

REGISTRATION OF PARTICIPANTS

Registration desk was open on Thursday, April 4 from 08:00 in front of Hall „Donji Dorćol“, Mona PLAZA hotel, as well as on Friday, April 5 and Saturday, April 6 at the same place. Daily updates on the workshop sessions and social events were available at the registration desk and through the specialy designed application for this Congress (https://play.google.com/store/apps/details?id=com.Mikrobiolozi). All participants and accompanying persons were kindly requested to wear their acreditational badges during the scientific sessions and workshop social events.

LANGUAGE

The official language of the congress was English.

SOCIAL EVENTS

A group photo was taken in front of Mona PLAZA hotel on Thursday, April 4th, at 11:50. The poster viewing session took place at Hall ‘Club’ of Hotel Palace, ground floor, located at Topličin Venac 23, Belgrade, on Friday from 17:45 to 19:15.”

INFORMATION FOR PRESENTERS

Oral presentations were held at the Hall “Donji Dorćol” and Hall “SCHONDA 4” , First floor of the hotel Mona PLAZA, Cara Uroša 62-64, Belgrade, from April 4th to 6th.

UMS 24° SERIES

04th - 06th April 2024

MONA PLAZA Hotel, **Belgrade**

XIII CONGRESS OF MICROBIOLOGISTS OF SERBIA

MIKROMED REGIO 5

WITH INTERNATIONAL PARTICIPATION

FROM BIOTECHNOLOGY TO HUMAN AND PLANETARY HEALTH

CONGRESS PROGRAMME

ORGANIZER :

SERBIAN SOCIETY FOR MICROBIOLOGY

SUPPORTED BY :

Federation of European Microbiological Societies

Republic of Serbia
MINISTRY OF SCIENCE, TECHNOLOGICAL DEVELOPMENT AND INNOVATION

UMS 24 PROGRAMME

Thursday, April 4, 2024

08:00-09:00 **Registration of participants**

Venue - Hall „DONJI DORĆOL" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

ORGANIZATIONAL OPENING REMARKS - **Ivica Dimkić, Dušan Kekić** & **Lazar Ranin**

08:45-09:30
Jelena Begović - Ministry of Science, Technological Development and Innovation
Ljubiša Stanisavljević - Dean of the Faculty of Biology of the University of Belgrade
Luciano Catani - Science Attaché at the Italian Embassy in Belgrade, Serbia
Vaso Taleski - FEMS Director of Events & Internationalization FEMS - 50 years of successful connecting people and sharing knowledge in microbiology worldwide

SESSION IA-1

MICROBIAL GENETICS, METAGENOMICS AND METAPROTEOMICS (PART 1) – MICROBIOMES AND RECENT DEVELOPMENTS & PROBIOTICS
Chaired by: **Maja Rupnik & Nataša Golić**

09:30-09:55 **Maja Rupnik (Slovenia):** The role of gut microbiota on infection with multiresistant bacteria

09:55-10:20 **Nataša Golić (Serbia):** The use of integrative multi-omics approach in cultivation and characterization of gut bacteria related to microbiota-gut-brain axis as a source for Next Generation Probiotics

10:20-10:45 **Vladimir Milivojević (Serbia):** The importance of probiotics and modulation of microbiota in gastroenterology

10:45-11:10 **Mirjana Rajilić-Stojanović (Serbia):** Modulation of human mycobiota as a tool for promoting health

11:10-11:35 **Marina Atanasković-Marković (Serbia):** Dangerous relations: bacteria, antimicrobial therapies, and allergic diseases

11:35-11:50 **Hristina Mitrović (Serbia, Offer. pres.):** From gut to lab: unlocking anti-inflammatory potential with GABA-producing bacteria

11:50-12:10 **AbelaPharm (INDUSTRY LECTURE) - Nada Tršić-Milanović (Serbia):** From microscope to shelf: How our research becomes strength

Venue - Hall „SCHONDA 4" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IB-1

INDUSTRIAL AND FOOD MICROBIAL BIOTECHNOLOGY (PART 1) - MICROBIAL VALORIZATION OF WASTES AND SECONDARY MATERIALS & BIOTECHNOLOGY AND SYNTHETIC MICROBIOLOGY
Chaired by: **Vera Karličić & Alexander Osmolovskiy**

09:30-09:55 **Alexander Osmolovskiy (Russian Federation):** Proteinases of filamentous fungi as activators of hemostatic system proteins: key properties and application

09:55-10:20 **Nemanja Mirković (Serbia):** Bacteriocins: past, current knowledge and future prospects
10:20-10:45 **Marina Jovanović (Serbia):** Valorization of psychobiotics and agri-food by products as functional ingredients
10:45-11:10 **Vera Karličić (Serbia):** Ecological services of beneficial microorganisms as a paradigm of sustainable agroecosystems
11:10-11:25 **Vincent Léguillier (France, Offer. pres.):** Structural optimization of an I-motif aptamer for the specific detection of *Staphylococcus aureus*
11:25-11:40 **Marija Duvnjak (Croatia, Offer. pres.):** Aerobic stability of the alfalfa silage

12:10-12:30 **COFFEE BREAK**
12:20-12:30 **GROUP PHOTO IN FRONT OF THE MONA PLAZA HOTEL**

Venue - Hall „DONJI DORĆOL" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IIA-1

ACTIVE IMMUNIZATION AS THE KEY ELEMENT IN INFECTION PREVENTION AND CONTROL (PART 1) - VIRAL VACCINES
Chaired by: **Maja Stanojević & Aleksandra Knežević**

12:30-12:55 **Maja Čupić (Serbia):** Vaccines for influenza and Covid 19 - what we need to know
12:55-13:20 **Aleksandra Knežević (Serbia):** HPV vaccines in the cancer prevention – recommendations and future prospective
13:20-13:45 **Maja Stanojević (Serbia):** Poliovirus eradication: challenges of containment
13:45-14:10 **Ivana Lazarević (Serbia):** Advances, challenges and novelties in HBV and HCV vaccines development
14:10-14:35 **Ana Banko (Serbia):** Development of new viral vaccines with a focus on the new RSV vaccine

Venue - Hall „SCHONDA 4" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IIB-1

ENVIRONMENTAL MICROBIOLOGY AND BIOTECHNOLOGY (PART 1) - HOST-MICROBE INTERACTIONS & TOWARDS A MORE SUSTAINABLE AGRICULTURE AND SOIL MICROBIAL LEGACY
Chaired by: **Ines Mandić Mulec & Ivica Dimkić**

12:30-12:55 **Ines Mandić Mulec (Slovenia):** Social strategies by beneficial bacterium *Bacillus subtilis*
12:55-13:20 **Vittorio Venturi (Italy):** Inter-species bacterial signaling in the plant microbiome
13:20-13:45 **Stéphane Compant (Austria):** Harnessing fungal-bacterial interactions to improve plant growth and health
13:45-14:10 **Ioannis Kampouris (Germany):** A consortium of plant-beneficial microorganisms mitigates drought effects on maize by aiding the recruitment of focal soil microorganisms in rhizosphere
14:10-14:25 **Dmitrii Deev (Slovenia, Offer. pres.):** Enhancing bioremediation efficiency: novel isolation techniques for microbial consortia in recalcitrant PAH-contaminated soils
14:25-14:40 **Ivana Galić (Serbia, Offer. pres.):** Soil microbiome diversity in maize-winter wheat crop rotation

14:40-15:30 **LUNCH BREAK**

Venue - Hall „DONJI DORĆOL" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

15:30-15:50 **EuroMedicina (INDUSTRY LECTURE) - Aleksa Jovanović (Serbia):** EXS2600 MALDI TOF - new possibilities

SESSION IIIA-1

ENVIRONMENTAL MICROBIOLOGY AND BIOTECHNOLOGY (PART 2) - TOWARDS A MORE SUSTAINABLE AGRICULTURE AND SOIL MICROBIAL LEGACY & PHYTOPATHOLOGY
Chaired by: **Jovana Grahovac & Aleš Lapanje**

15:50-16:15 **Aleš Lapanje (Slovenia):** Harnessing spatial microbiome dynamics for cutting-edge environmental biotechnology

16:15-16:40 **Đorđe Bajić (The Netherlands):** Using fitness landscapes to engineer optimal function in microbial communities

16:40-17:05 **Matjaž Hladnik (Slovenia):** Comparative analysis of phyllosphere microbiota in olive leaf spot disease

17:05-17:30 **Jovana Grahovac (Serbia):** Microbial biomass production and application within biorefinery concept

17:30-17:45 **Marko Vincenković (Croatia, Offer. pres.):** Preparation, characterization and application of copper microparticles in controlling of phytopathogenic fungi

Venue - Hall „SCHONDA 4" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IIIB-1

PANEL SESSION - INFECTIONS IN PATIENTS ON IMMUNOMODULATION AND IMUNOSUPRESIVE THERAPIES
Moderator: **Dušan Kekić**

15:50-17:45

PANELISTS:

Gordana Petrović (Serbia)
Tamara Knežević (Serbia)
Borko Gobeljić (Serbia)
Aleksandra Plavšić (Serbia)
Ana Banko (Serbia)

PRESENTING CASES:

Tijana Đerić (Serbia): Occurrence of pneumonia in a patient on biological therapy due to TRAPS

Olga Odanović (Serbia): Infections in patients with inflammatory Bowel disease on biologic therapy: Challenges in management

Ljubica Matić (Serbia): Biological therapy and infections in hematopoietic stem cell transplantation

Uroš Karić (Serbia): A series of rare infectious complications in an immunosuppressed patient with SLE

17:45-18:10 **COFFEE BREAK**

Venue - Hall „DONJI DORĆOL" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IVA-1

INDUSTRIAL AND FOOD MICROBIAL BIOTECHNOLOGY (PART 2) - FOOD MICROBIOLOGY
Chaired by: **Mirna Mrkonjić Fuka & Konstantinos Papadimitriou**

- **18:10-18:35** **Tamar Sachaneli (Georgia):** Microbial diversity of Georgian artisanal cheese production
- **18:35-19:00** **Konstantinos Papadimitriou (Greece):** Multi-omics approaches to characterize the microbiome of certain greek artisanal fermented foods and wines
- **19:00-19:25** **Mirna Mrkonjić Fuka (Croatia):** Fermented food ecosystems - a treasure chest of untapped microbial potential
- **19:25-19:40** **Marija Todorovska Ivkovikj (North Macedonia, Offer. pres.):** Isolation and characterization of yeasts from Macedonian wineyards for production of Macedonian unique wine

Venue - Hall „SCHONDA 4" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IVB-1

ACTIVE IMMUNIZATION AS THE KEY ELEMENT IN INFECTION PREVENTION AND CONTROL (PART 2) - VIRAL VACCINES
Chaired by: **Danijela Miljanović & Ivana Lukić**

- **18:10-18:35** **Danijela Miljanović (Serbia):** MMR vaccine and seroprevalence of measles, mumps and rubella IgG antibodies among young medical students in Serbia
- **18:35-19:00** **Ivana Lukić (Serbia):** mRNA vaccine manufacturing – challenges in plasmid DNA cloning vector design
- **19:00-19:15** **Marko Janković (Serbia, Offer. pres.):** Human cytomegalovirus oncoprotection across diverse populations, tumour histologies, and age groups: the relevance for prospective vaccinal therapy

Friday, April 5, 2024

- **08:00-09:00** **Registration of participants**

Venue - Hall „DONJI DORĆOL" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IA-2

MULTI DRUG AND PAN DRUG RESISTANCE & HEALTH MICROBIOLOGY AND BIOTECHNOLOGY (PART 1)
Chaired by: **Ina Gajić & Paul Cos**

- **09:00-09:25** **Paul Cos (Belgium):** Challenges and lessons learned from antimicrobial research projects
- **09:25-09:50** **Ina Gajić (Serbia):** Genomic epidemiology of carbapenem-resistant *Pseudomonas aeruginosa*

- **09:50-10:15** **Katarina Novović (Serbia):** *Acinetobacter baumannii* resistant to last line antibiotics: an emerging threat in Western Balkan
- **10:15-10:40** **Dušan Milivojević (Serbia):** From soil to lab: Exploring toxicology with *Caenorhabditis elegans*
- **10:40-10:55** **Miloš Jovićević (Serbia, Offer. pres.):** High-level resistance of carbapenem-resistant *Klebsiella pneumoniae* to novel β-lactam-β-lactamase inhibitor combinations in clinical settings in Serbia
- **10:55-11:20** **Jose Alexander (USA) - *online*:** Understanding antimicrobial resistance for testing and treatment strategies
- **11:20-11:40** **bioMérieux (INDUSTRY LECTURE) - Snežana Jovanović (Serbia):** Syndromic PCR or how to solve diagnostic Rubik's cube easily

Venue - Hall „SCHONDA 4" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IB-2

MICROBIAL GENETICS, METAGENOMICS AND METAPROTEOMICS (PART 2) - MICROBIOMES AND RECENT DEVELOPMENTS & MICROBIAL GENOMES AND THEIR EVOLUTION

Chaired by: **Livia Leoni & Gergely Maróti**

- **09:00-09:25** **Livia Leoni (Italy):** Role of stringent response in *Pseudomonas aeruginosa* virulence
- **09:25-09:50** **Gergely Maróti (Hungary):** Genome level investigation of inter-kingdom microbial interactions
- **09:50-10:15** **Elena Perrin (Italy):** Insights into the evolution of multipartite genomes in *Proteobacteria*
- **10:15-10:40** **Svetlana Ugarčina Perović (Italy) - *online*:** Challenges in metagenomic annotation of antibiotic resistome
- **10:40-10:55** **Marko Panić (Serbia, Offer. pres.):** Exploring *E. coli*-based expression of genetically inactivated tetanus toxin for vaccine development
- **10:55-11:10** **Allwin Mabes Raj (Slovenia, Offer. pres.):** Mer B (organomercurial-lyase) mediated quartz crystal microbalance (QCM) based methylmercury detection

11:40-11:55 **COFFEE BREAK**

Venue - Hall „DONJI DORĆOL" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IIA-2

ACTIVE IMMUNIZATION AS THE KEY ELEMENT IN INFECTION PREVENTION AND CONTROL (PART 3) - BACTERIAL VACCINES

Chaired by: **Tamara Kastrin & Nataša Opavski**

- **11:55-12:20** **Tamara Kastrin (Slovenia):** The importance of vaccination and national surveillance of invasive bacterial diseases and whooping cough in Slovenia
- **12:20-12:45** **Nataša Opavski (Serbia):** Do we need higher valency pneumococcal conjugate vaccines in Serbia?
- **12:45-13:10** **Marko Veljković (Serbia):** Vaccination against pertussis in Serbia: past, current challanges and future perspectives
- **13:10-13:35** **Nevena Jovičić (Serbia):** Complications of pneumococcal pneumonia in children
- **13:35-14:00** **Aleksandar Sovtić (Serbia):** Respiratory infections in children – over the horizon

Venue - Hall „SCHONDA 4" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IIB-2

ENVIRONMENTAL MICROBIOLOGY AND BIOTECHNOLOGY (PART 3) - BIODETERIORATION OF MATERIALS: EXTREME ENVIRONMENTS & ANTIMICROBIAL RESISTANCE: A ONE HEALTH CHALLENGE
Chaired by: **Cecilia Flocco & Nikola Unković**

- **11:55-12:20** **Cecilia Flocco (Germany):** New perspectives for microbiology and biotechnology in cultural heritage research
- **12:20-12:45** **Nikola Unković (Serbia):** Research into the application of bacterial-based bioformulations in the conservation of fungal-deteriorated works of art in Serbia
- **12:45-13:10** **Nikolina Udiković Kolić (Croatia):** Environmental spread of antibiotic resistance – the role of industrial, agricultural and municipal waste
- **13:10-13:35** **Stoimir Kolarević (Serbia):** Impact of untreated wastewaters on the microbiological water quality of the Danube river and its tributaries in Serbia
- **13:35-14:00** **Dragana Predojević (Serbia):** Phytoplankon in small water bodies

14:00-15:00 **LUNCH BREAK**

Venue - Hall „DONJI DORĆOL" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

15:00-15:20 **Pfizer (INDUSTRY LECTURE) - Ivana Ćirković (Serbia):** Together in the fight against AMR, from immunization and diagnostics to targeted therapy

SESSION IIIA-2

ALTERNATIVE APPROACHES IN ANTIMICROBIAL CONTROL (PART 1) - BACTERIOPHAGES APPLICATIONS
Chaired by: **Mariagrazia Di Luca & Goran Vukotić**

- **15:20-15:45** **Mariagrazia Di Luca (Italy):** Unveiling the Janus-Face of Bacteriophages: A dual perspective on antibacterial therapy
- **15:45-16:10** **Hugo A. M. de Oliveira (Portugal):** Novel bacteriophage-based depolymerase strategies to control *A. baumannii*
- **16:10-16:35** **Goran Vukotić (Serbia):** Bacteriophages of multidrug-resistant nosocomial pathogens – Belgrade experience
- **16:35-17:00** **Luís D. R. de Melo (Portugal):** Phage-host interaction with cells in different metabolic states: A *S. epidermidis* case
- **17:00-17:15** **Sonja Gostimirović (Serbia, Offer. pres.):** Non-tailed icosahedral phages as antibacterial agents

Venue - Hall „SCHONDA 4" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IIIB-2

PANEL SESSION - INTRAHOSPITAL AND EMERGING INFECTIONS
Moderator: **Dušan Kekić**

15:20-17:15 PANELISTS:

Miodrag Milenović (Serbia)
Novica Nikolić (Serbia)
Jelena Jordović (Serbia)

PRESENTING CASES:

Marija Rajković (Serbia): Fulminant necrotizing fasciitis: A fatal outcome. Case series

Elena Đukić (Serbia): Unraveling the mystery: A case of septic shock with unknown orgin

Ana Mirković (Serbia): A case report of *Staphylococcus aureus* prosthetic valve endocarditis in patient with Randu – Osler – Weber syndrome

Martina Jug (Serbia): Aspergillosis - diagnostic and therapeutic challenges

17:45-19:15 **POSTER VIEWING SESSION - HALL CLUB OF THE HOTEL PALACE, GROUND FLOOR, TOPLIČIN VENAC 23, BELGRADE**

Saturday, April 6, 2024

08:30-09:30 **Registration of participants**

Venue - Hall „DONJI DORĆOL" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IA-3

MICROBIAL GENETICS, METAGENOMICS AND METAPROTEOMICS (PART 3) - MICROBIOMES AND RECENT DEVELOPMENTS & MICROBIAL GENOMES AND THEIR EVOLUTION
Chaired by: **Tamara Janakiev & Alfonso Esposito**

09:30-09:55 **Nađa Nikolić (Serbia):** Microbiomes in oral carcinomas

09:55-10:20 **Alfonso Esposito (Italy):** Next-Generation Sequencing and Bioinformatics: How does microbiology benefit from cutting edge technologies?

10:20-10:45 **Tamara Janakiev (Serbia):** Plant microbiomes: from diversity to healthy crops

10:45-11:00 **Maurizio Zotti (Italy, Offer. pres.):** Microbiome associated with mycelial activity of three species of *Basidiomycetes*: Fairy rings in the gardens of the Royal Palace of Caserta (Italy)

11:00-11:15 **Daria Tsibulskaia (Serbia, Offer. pres.):** Description of a new potential aggregation factor from the *Streptococcus thermophilus* genome

11:15-11:40 **Jose Alexander (USA) - *online*:** Implementation of a rapid, cost-effective, and clinically focused NGS solution

Venue - Hall „SCHONDA 4" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IB-3

HEALTH MICROBIOLOGY AND BIOTECHNOLOGY & ALTERNATIVE APPROACHES IN ANTIMICROBIAL CONTROL (PART 2)
Chaired by: **Lidija Đokić & Jon Salmanton-García**

09:30-09:55 **Marina Šantić (Croatia):** *Legionella pneumophila* - journey from the environment to human macrophages

09:55-10:20	**Jon Salmanton-García (Germany):** Overview on the current situation on epidemiology and management of IFI and future perspectives
10:20-10:45	**Ivana Čolović Čalovski (Serbia):** Diagnosis of intestinal helminth infections: strengths and limitations
10:45-11:10	**Eleonora Dubljanin (Serbia):** Biomarker guided antifungal therapy: a current state of laboratory mycology and antifungal management
11:10-11:35	**Lidija Đokić (Serbia):** New approaches in the treatment of chronic bacterial infections

11:40-12:10 **COFFEE BREAK**

Venue - Hall „DONJI DORĆOL" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IIA-3

ALTERNATIVE APPROACHES IN ANTIMICROBIAL CONTROL (PART 3) - BIOTECHNOLOGICAL APPROACH OF USING NATURAL PRODUCTS & NANOTECHNOLOGY IN MICROBIOLOGY
Chaired by: **Marina Soković & Ivana Gobin**

12:10-12:35	**Marina Soković (Serbia):** Microfungi as a target and source of valuable compounds
12:35-13:00	**Ivana Gobin (Croatia):** Biofilms in premise plumbing systems - current challenges and potential solutions
13:00-13:25	**Marina T. Milenković (Serbia):** Herbal products as an alternative to antibiotics: application possibilities and limitations
13:25-13:50	**Tatjana Stević (Serbia):** Biocontrol activity of plant products against plant pathogens
13:50-14:05	**Miroslav Dinić (Serbia, Offer. pres.):** Host-microbiota interplay regulates epithelial barrier function and wound healing

Venue - Hall „SCHONDA 4" of the MONA PLAZA Hotel, Cara Uroša 62-64, First floor

SESSION IIB-3

WORKSHOP "NEXT GENERATION SEQUENCING (NGS) IN MICROBIOLOGY "
Moderators: **Valentina Đorđević & Ivana Morić**

12:10-12:35	**Valentina Đorđević (Serbia):** Center for Genome Sequencing and Bioinformatics
12:35-13:00	**Ivana Morić (Serbia):** Introduction to NGS technologies
13:00-13:25	**Mirjana Novković (Serbia):** Genome sequence diversity of SARS-CoV-2 in Serbia during pandemic
13:25-13:55	**Nada Stanković & Ivana Galić (Serbia):** Metagenomic studies

14:15-14:30 **ORGANIZATIONAL CLOSING REMARKS**

INDUSTRIAL AND FOOD MICROBIAL BIOTECHNOLOGY

IL1

PROTEINASES OF FILAMENTOUS FUNGI AS ACTIVATORS OF HEMOSTATIC SYSTEM PROTEINS: KEY PROPERTIES AND APPLICATION

Alexander Osmolovskiy[1]
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Contact: aosmol@mail.ru

Most available drugs for the treatment and timely prevention of thromboembolic complications have insufficient specificity of action and high cost, and their use in the treatment of these diseases is associated with the risk of side effects. Therefore, the actual problem of modern biotechnology is to find and develop new methods for producing drugs, in particular, based on proteolytic enzymes of microscopic fungi. Huge interest for practical medicine has proteinases of micromycetes with high fibrinolytic activity and the ability to activate some of the hemostatic system proteins by their limited proteolysis. Recent studies at Moscow State University showed that fungi are able to secrete proteases, highly cleaving fibrinogen and fibrin and activating protein C, plasminogen, prekallikrein and factor X - core proteins of the hemostatic system, changing the content of which in the blood stream leads to various diseases. *Aspergillus ochraceus* is the producer of proteinases – activators of protein C and factor X. Their properties are similar to proteinases-activators derived from the venom of the southern American copperhead snake and Russell's viper, respectively, and which used for the diagnosis of these proteins is currently. The character of the calibration curves for determination in plasma protein C and factor X levels in case of usage proteinase of *Aspergillus ochraceus* is very close to the character of commercial analogs' schedules (based on snake venom proteases). Applicability of the calibration curves was checked by suitable reactions with plasmas with artificially reduced level of protein C and factor X or deficient plasmas. *Aspergillus terreus* is the producer of proteases with proved prekallikrein-activation activity. The development of a diagnostic kit for the determination of plasma prekallikrein formed on the basis of these micromycetal proteases is being developed. Fungal proteinases may be used as a promising alternative in diagnostics for targeted plasma proteins determination.

KEYWORDS: *Aspergillus*; proteolytic enzymes; protein C activators

IL2

MICROBIAL BIOMASS PRODUCTION AND APPLICATION WITHIN BIOREFINERY CONCEPT

Jovana Grahovac[1], Vanja Vlajkov[1], Mila Grahovac[2] and Ivana Pajčin[1]
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Microbial biomass represents an alternative protein source that has long been proposed as a solution to food scarcity and that can be more sustainable than conventional food protein sources. Microbial inoculants possess the capacity to enhance nutrient availability, uptake, and support the health of soil and plants to promote sustainable yield. Also, microbial biomass has significant role in bioremediation technologies relying on the ability of microorganisms to degrade organic contaminants. The lack of full commercialization of microbial biomass for the aforementioned application is attributed to the high costs of biotechnological production. The usage of alternative complex media based on agro and food industry effluents could be beneficial from the point of view of economic efficiency of the designed bioprocesses. Also, harmonizing the production of microbial biomass with the principles of product-driven biorefineries would improve sustainability of the production processes. Industrial sector is often unaware of the possibilities and the economic value held in waste streams but very high tipping fees for waste disposal and climate change levies have already stimulated innovation and action in by-product reuse. Still, there is a gap between potential embedded in waste and the recognition of biotechnology as a powerful tool offering promising solutions for waste valorization. The solution represents usage of biotechnological processes for treatment of industrial wastewaters, simultaneously obtaining value-added products by microorganisms using residual substances held in the industrial waste streams. This could also present a way to recycle water and decrease industrial fresh water consumption which would reduce the pressure on water resources. The aim of this work is systematization of data on developed solutions for microbial biomass production within biorefinery concept.

KEYWORDS: microbial biomass; biorefinery; waste valorization

ACKNOWLEDGEMENT: The authors gratefully acknowledge the financial support of Ministry of science, technological development and innovation (451-03-65/2024-03/200134 and 451-03-66/2024-03/200134).

IL3

MULTI-OMICS APPROACHES TO CHARACTERIZE THE MICROBIOME OF CERTAIN GREEK ARTISANAL FERMENTED FOODS AND WINES

Konstantinos Papadimitriou[1], Marina Papadelli[2] and John Kapolos[2]

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Many different artisanal fermented foods and wines are produced in Greece. Both the mountainous terrain of the mainland and the high number of islands support the presence of geographically discrete areas. These areas may constitute isolated ecological niches that lead to the production of unique agricultural raw materials which are often used to produce artisanal fermented foods and wines with unique organoleptic characteristics. Some of these foods and wines have been studied over the years to an extent, but the majority are still produced more or less in an empirical manner. The advent of high-throughput omics methods over the last two decades has provided us with the opportunity to produce vast amounts of microbiological and/or analytical data for any type of fermented food even those that have not been in the focus of scientific studies before. Here we will present the application of a multiomics strategy to study certain PDO dairy products, table olives and musts for wines. In a first step we employed amplicon sequencing and shotgun metagenomics which provided us with a detailed picture of the fermenting ecosystem. This data allowed us to use culture-based analysis in order to isolate some of the bacteria and yeast that were present in the product and identify them with MALDI-TOF MS. We also used GC-IMS analysis to rapidly identify at least some of the volatile metabolites that contribute to the aroma and flavor of the products. Finally, FT-IR was used to acquire a very rapid fingerprint of the overall chemical composition of the fermented foods and we found that we could distinguish the products at the producer level in more than one occasions. This data will allow us to better understand the production of the particular products and to isolate novel starters or adjuncts with improved properties.

KEYWORDS: sfela; kalamata olives; nemea wine

ACKNOWLEDGEMENT: This research has been co-financed by the European Regional Development Fund (ERDF) and Greek National Funds through the Operational Program "Peloponnese 2021-2027" (MIS: 6001408).

IL4

VALORIZATION OF PSYCHOBIOTICS AND AGRI-FOOD BY PRODUCTS AS FUNCTIONAL INGREDIENTS

Marina Jovanović[1], Petar Vojvodić[2] and Dragana Mitić Ćulafić[3]
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In Serbia, the use of fermented products has a long tradition, but fermentation with psychobiotic strains (PS) is an innovative strategy. Psychobiotics refer to viable health-promoting microorganisms (probiotics), cell-free probiotic extracts (paraprobiotics), substances selectively used by the PS to improve host health (prebiotics), and microbiota-targeted interventions that manipulate the gut-microbiota-brain axis and have positive effects on neurological functions. Additionally, fermented products supplemented with agro-food by-products (AFBP) could serve as antimicrobials and prebiotics, and therefore suppress the growth of pathobionts, increase the viability of lactic acid bacteria (LAB) and consequently increase the amount of synthesized neuroactive LAB metabolites affecting a brain function and mood. Our studies confirmed that coagulated fresh soft quark cheese fermented with *Limosilactobacillus reuteri* and gourmet mushroom powders and yogurts with *Ganoderma lucidum* residues could serve as PS carrier and additionally promote biological effects. A high abundance of LAB has been maintained (>log 7.64±0.23 cheese samples; >9.18±0.83 log CFU/mL yogurt samples). Yogurt samples exert anti-*E. coli* effect on patogenic *E. coli* 0157:H7 and *E. coli* ATCC 35218, while cheeses supported high viability of probiotic *E. coli* Nissle 1917 (>7.11 log CFU/mL). Further on, cytotoxicity to colon cancer cells, HCT116 was investigated and inhibition of cell viability was up to 30.96% for cheeses and up to 25% for yogurts. Sensory evaluation revealed high scores for cheese samples. PS and AFBP products represent the food of a new generation, so it is of a great importance to gain the consumers' trust. Surveying 168 respondents (52.7% female, 47.3% male) it has been shown that 65.9% would try PS based products, 51.5% would not try zero waste edibles, while 52.1% exerts neophobia towards this kind of food. The neophobia should first be addressed. Education is needed on both subject, nutritional psychiatry and zero waste, before designing and marketing PS and AFBP ferments.

KEYWORDS: zero waste; psychobiotics; fermented beverages; cheeses

ACKNOWLEDGEMENT: This research was funded by the Ministry of Education, Science, and Technological Development of the Republic of Serbia, contract number 451-03-47/2023-01/200051 and 451-03-47/2023-01/200178.

IL5

FERMENTED FOOD ECOSYSTEMS - A TREASURE CHEST OF UNTAPPED MICROBIAL POTENTIAL

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Spontaneously fermented artisan cheese and sausages are complex microbial ecosystems, recognized and appreciated as extremely valuable food products. They are characterized by unique aroma that is a consequence of complex microbial metabolic activities and interactions. Spontaneously fermented foods of animal origin can be the source of new presumptive starter or bioprotective strains, but they can also promote the growth of undesirable microbes due to the lack of pasteurization, the variability of ingredients and additives used, or the production conditions. Close monitoring of bacterial communities and reliable identification of beneficial and pathogenic microbiota is therefore crucial to ensure the quality and safety of spontaneously fermented foods. Traditionally, the presence of microorganisms in food is analyzed using culture-based methods. However, it is well known that these methods cannot reveal true bacterial richness, as artisanal foods can harbor complex consortia of microorganisms, only a small proportion of which can be easily isolated. The additional problem we face is the poor selectivity of "selective" agar media. The mass sequencing approach has been described as a promising tool as it can detect unique taxa in food matrix and thus track the rapid changes in the structure of microbial communities during the fermentation and ripening. However, since no method in microbiology is free from bias, a combined toolbox is proposed to analyze the changes in microbial communities and identify dominant members during the maturation of spontaneously fermented foods. All these aspects will be discussed based on our own research on the most popular Croatian artisanal hard cheeses and dry wild boar sausages. Particular attention will be paid to the search for "micro-treasures" - beneficial microbiota - that can be used for the production of high-quality sausages, with the focus on increasing their survival rate and tracking them after application in the food matrix.

KEYWORDS: spontaneously fermented food; cheese and sausages; complex microbial communities; beneficial microbiota; starter cultures

IL6

BACTERIOCINS: PAST, CURRENT KNOWLEDGE AND FUTURE PROSPECTS

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Bacteriocins were first identified almost 100 years ago as cationic, ribosomally synthesized, antimicrobial peptides that act by pore formation and disruption of the integrity of the target cell membrane, ultimately leading to cell death. As a group of different antimicrobial peptides, bacteriocins are known as inhibitors against closely related organisms such as Gram-positive and Gram-negative bacteria, especially lactic acid bacteria (LAB). They are divided into 3 main classes based on the last categorization. Considering the limited use of antibiotics in food production, much research is focused on the invention of new and more effective bacteriocins. LAB-derived bacteriocins such as nisin, pediocin PA-1, mersacidin, mutacin and lacticin are mainly used in the food industry as preservatives that can prevent the growth of *C. botulinum, E. faecalis*, *L. monocytogenes, S. aureus* and other food-borne pathogens. On the other hand, antibiotics play an important role in the prevention and treatment of diseases in animals and humans. However, due to the adverse effects of some antibiotics, the emergence of antibiotic-resistant MDR (multi-drug resistant) strains has recently become a major concern. For this reason, there is a need to develop a new strategy that could control or eliminate antibiotic-resistant microorganisms. Considering the fact that bacteriocins are very active in nanomolar concentrations, they are increasingly becoming a potential replacement for antibiotics in the control of pathogenic bacteria. It is assumed that these antimicrobial peptides offer more protection than antibiotics and have no side effects. According to the latest study results, the selection of bacteriocin or bacteriocin-producing strains suitable for use in the food industry or in medicine is very important. This is a direct indication that research in this area must continue in order to provide safe products with better nutritional properties and at the same time find an answer to resistant pathogens in medicine.

KEYWORDS: bacteriocins; lactic acid bacteria; food borne pathogen; multidrug-resistant

ACKNOWLEDGEMENT: This research was funded by the Ministry of Science and Technological Development and Innovation of the Republic of Serbia through agreement 451-03-65/2024-03/200116

IL7

MICROBIAL DIVERSITY OF GEORGIAN ARTISANAL CHEESE PRODUCTION

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Georgian traditional PDO cheeses Tushuri Guda and Shushvela are distinguished by special peculiarities of technology and gustative features. Georgian small producers in high mountain regions keep up the tradition. The microbial constitution of cheese is one of the key factors for its taste and aroma formation. The isolation of the indigenous microbiota is very significant to studying biodiversity. The aim of the presented study was the establishment of the dominant components of the microbiota of Tushuri Guda cheese and Shushvela and the selection of the prospective strains according to their morphological, physiological, biochemical, and probiotic characteristics. Endemic bacteria of lactic- and propionic acid bacteria characteristic of Guda-cheese and Shushvela have been isolated. The probiotic characteristics (antibacterial activity, tolerance towards bile and acid) and biotechnologically significant properties (proteolytic, lipolytic, and acidification activities) of the bacteria have been studied. The dominant components of the microbiota of all studied samples were lactic acid bacteria (*Lactobacillus* and *Lactococcus*), as well as propionic acid bacteria and yeasts. The prospective probiotic strains of propionic- and lactic acid bacteria were revealed during the investigation of tolerance towards 12 pathogenic and conditionally pathogenic test cultures, as well as by tolerance towards bile and low acidity (pH 2). According to different morphological (shape of cells and colonies, Gram staining), physiological (temperature, concentration of NaCl) and biochemical (catalytic activity, fermentation of different carbon sources, urease activity, arginine hydrolysis, aesculin hydrolysis, hemolysis, gelatin hydrolysis) properties following species were identified: *Lb. acidophilus*, *Lb. helveticus*, *L. lactis.* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. mesenteroides* subsp. *mesenteroides*, *Lb. rhamnosus*, *P.acidi-propionici, S. thermophilus, and Lactobacillus delbrueckii ssp. bulgaricus.* The revealed species distinguished by probiotic and technological properties may be used for further application in food biotechnology.

KEYWORDS: traditional PDO cheese; indigenous microbiota; shushvela cheese; lactic acid bacteria; propionic acid bacteria

OF8

MER B (ORGANOMERCURIAL-LYASE) MEDIATED QUARTZ CRYSTAL MICROBALANCE (QCM) BASED METHYLMERCURY DETECTION

A. F. P. Allwin Mabes Raj[1, 2, 3], Tomaz Rijavec[1], Tayebeh Sharifi[1], Igor Živković[1, 2], Polona Klemenčič[1], Adna Alilović[1], Ermira Begu[1], Milena Horvat[1, 2], Aleksandra Lobnik[3, 4], Aljoša Košak[3,4] and Aleš Lapanje*[1]

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Mercury is a highly toxic and mobile element that has had a pronounced and adverse effect on organisms. Accordingly, bacteria have evolved mer operons to meliorate the toxic action of different chemical forms of mercury. The bacterial mercury detoxification system contains two proteins, organomercurial lyase (MerB) and mercuric ion reductase (MerA). MerB specifically catalyses the protonolysis of the carbon-mercury bond of methylmercury (MeHg), resulting in the formation of a reduced carbon compound and inorganic ionic mercury (Hg^{2+}). Since MerB is a highly specific organomercurial lyase, we plan to use its Met-Hg-specific binding characteristics as a sensing/receptor component of the sensor. We intend to develop a Quartz crystal microbalance sensor (QCM) for detecting MeHg through the changes in the resonant frequency of a quartz crystal induced by the redox potential of Hg-bound-MerB after cleaving MeHg. On binding of MeHg onto MerB, we would determine the frequency and bandwidth of the crystal resonance. To achieve that, we have prepared an expression system that will enable us to obtain enough highly active MerB enzymes. The expressed MerB enzyme with his-tag was purified and it showed High Hg-bound onto the MerB after cleaving from MeHg for preparing the Met-Hg-specific sensor. For the reporter system, we have prepared an SPE (Screen-printed gold electrode) and functionalized it with thiol followed by NTA-Ni^{2+} (Nitriloacetic acid with Nickel) for specific Histag MerB proteins binding and completed the initial characterisation. We are planning to characterize further proteins and the proteins immobilized in electrodes to find the ideal conditions for binding MeHg onto MerB for MeHg detection. MerB has been prepared as a specific bioreceptor for MeHg sensing, and Au@ NTA-Ni^{2+} electrodes were prepared as a platform for immobilization of Mer B and Mer A. We are planning to characterize furthermore on proteins immobilized in electrodes.

KEYWORDS: methylmercury detection; sensing: MerB (organomercurial-lyase); quartz crystal microbalance

ACKNOWLEDGEMENTS Slovenian research agency (grant no.: P2-0150), EUIA project Applause (UIA02-228), GMOS-Train Program European Union Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no. 860497, European Commission, grant agreements P1-0143, 826312, 952379 and 101060211.

OF9

AEROBIC STABILITY OF THE ALFALFA SILAGE

Marija Duvnjak[1], Vida Vertuš[1], Mirna Mrkonjić Fuka[1], Manuela Zadravec[2] and Kristina Kljak[1]
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During the feedout phase and aeration, the activity of aerobic microorganisms leads to a deterioration of the silage, which is indicated by the degradation of lactic acid, nutrient losses and an increase in silage temperature. A higher content of volatile fatty acids (VFA), which is achieved by the addition of obligate heterofermentative lactic acid bacteria (LAB) during silage preparation, increases the aerobic stability of the silage. The aim of this study was to investigate the microbiological characteristics and aerobic stability parameters of alfalfa (*Medicago sativa* L.) silage, treated with LAB inoculant during a seven-day aeration period. The silage treatment included silages inoculated with *Pediococcus pentosaceus, Lentilactobacillus buchneri* and *Lentilactobacillus hilgardii* (INOC) and a control (CONT) without inoculant. The silage samples were analysed in five repetitions for aerobic stability, the level of LAB, yeasts, moulds and butyric acid-producing spore forming bacteria (BAB) as well as for fermentation parameters (lactic acid, acetic acid, propionic acid, butyric acid, ethanol). The silages tested showed stable but diverse aerobic conditions during aeration, with the CONT silages showing three times higher lactic acid levels compared to the INOC silages ($P<0.05$), indicating homofermentative activity in CONT. Application of the inoculant had a positive effect on acetic acid content at aeration (47 vs. 30 g/kg DM, $P<0.05$) and propionic acid content (2.01 vs. 0.76 g/kg DM; $P<0.05$), with no negative effect on butyric acid (both INOC and CONT below 5 g/kg DM) and ethanol content (both INOC and CONT below 1.5 g/kg DM). The use of the inoculant resulted in higher LAB counts during aeration (about 1.16x more LAB). Yeasts and moulds remained below average in all silages (yeasts < 3.1 log10 CFU/g, moulds < 4.1 log10 CFU/g). This study shows that the use of a heterofermentative LAB inoculant is an effective method for improving the aerobic stability of alfalfa silage

KEYWORDS: aerobic stability; alfalfa silage; lactic acid bacteria inoculant

OF10

ISOLATION AND CHARACTERIZATION OF YEASTS FROM MACEDONIAN WINEYARDS FOR PRODUCTION OF MACEDONIAN UNIQUE WINE

Marija Todorovska-Ivkovikj[1,2], Igor Pavlovski[1,2], Dzoko Kungulovski[2] and Natalija Atanasova-Pancevska[2]
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By studying indigenous yeast populations, this study can gain insights into the unique flavors, aromas, and fermentation characteristics they contribute to local food and beverage production. This knowledge can have applications in various industries, such as brewing, winemaking, and breadmaking. The goal of our research is to isolate, identify, and characterize wild yeasts from the surface of grapes from different parts of NR Macedonia and use them for the production of Macedonian unique wine. Once the yeast strains were isolated, they were characterized by studying their morphological, physiological, biochemical and oenological properties. This includes examining their growth patterns, metabolic capabilities, and tolerance to different environmental conditions. Additionally, genetic analysis were performed to understand the genetic diversity and relationships among the isolated yeast strains. Furthermore, the isolates were plated on YPB medium. From the samples collected, 173 putative yeast strains were isolated. Each isolate was testet for ethanol and also for temperature, osmotic preasure and $S03^{2-}$ tolerance. 159 of isolates have tolerance to high osmotic preasure. Among them 53 strains have tolerance to high alcohol concentrations up to 15%. All of yeast strains showed to be resistant to 150 mg/L $S03^{2-}$. 60% of isolates produce high amount of CO2. Among the isolates studied, all yeast strains are thermotolerant. The present study allowed the isolation and characterization of V-59 and V-63 isolates. These isolates could contribute for the improvement of the wine quality and also could be used to create an identity for the wine produced in Macedonia.

KEYWORDS: yeasts; unique wine; indigenous; winemaking; tolerance

OF11

PREPARATION, CHARACTERIZATION AND APPLICATION OF COPPER MICROPARTICLES IN CONTROLLING OF PHYTOPATHOGENIC FUNGI

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The global demand for sustainable and environmentally friendly solutions in agriculture has encouraged the exploration of innovative methods in the field of plant protection. One of the innovative methods for the preparation of novel agroecological products designed to enhance plant protection is the green chemistry process of encapsulation. Encapsulation involves the capture of active substances within protective matrices, allowing for controlled release and improved efficacy. This method not only ensures targeted delivery of plant protection agents but also minimizes environmental impact and optimizes resource utilization. Copper, a bioactive element, has long been recognized for its potent antifungal properties, making it a cornerstone in plant protection strategies against phytopathogenic fungi. While copper stands as a vital bioactive component for protecting plants against phytopathogenic fungi, its application comes with the drawback of potential accumulation in the soil. It is precisely for this reason that the encapsulation of different concentrations of copper ions (2,3 and 4%) in alginate-chitosan biopolymer microparticles was carried out by the process of ion gelation. The prepared microparticles were characterized physico-chemically and it was determined that the main interactions between alginate and copper ions are of an electrostatic nature with a significant influence of hydrogen bonds. Their size, shape, surface morphology as well as the kinetics of the release of copper ions over time, which are carried out by the process of dialysis in contact with the aqueous medium were determined. To investigate the influence of different concentrations of biopolymeric copper microparticles selected are two phytopathogenic fungi, *Cercospora beticola* and *Botrytis cinerea*, and a pseudofungus *Phytophthora ramorum*. The prepared formulations influenced the growth of *Cercospora beticola* mycelia and the formation of sporangia of *Phytophthora ramorum*. Germination of conidia of *Botrytis cinerea* was not stopped on any of the used concentration.

KEYWORDS: encapsulation; phytopathogenic fungi; copper alginate; microparticles; plant protection

OF12

STRUCTURAL OPTIMIZATION OF AN I-MOTIF APTAMER FOR THE SPECIFIC DETECTION OF *STAPHYLOCOCCUS AUREUS*

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Staphylococcus aureus is a dangerous common foodborne and opportunistic pathogen, leading to a range of severe diseases with a significant impact on morbidity and mortality in humans and animals. This bacterium has a notable ability to quickly adapt and develop resistance to its environment, producing an array of virulence factors that facilitate immune invasion, tissue adhesion or host cell injury. To reduce risks and control disease progression, various methods have been developed for the detection of *S. aureus* including enumeration, PCR, RT-PCR, immunoassays methods, MALDI-TOF. These techniques are labor-intensive, time- consuming and results are sometimes difficult to interpret. To overcome the shortcomings of reference methods for pathogen detection, the development of biosensors has grown exponentially in recent decades. A biosensor is an analytical device containing a biorecognition element. In our work, we investigated the SA61 aptamer selected to bind to *S. aureus* cells in order to optimize its stability and increase affinity which is necessary for efficient detection of *S. aureus* directly in biological media such as milk and serum. We focused on the structural optimization of an intercalated-motif of the SA61 aptamer. By employing circular dichroism, fluorescent spectroscopy, melting temperature and NMR spectroscopy a comprehensive description of its stability, structure and topology was obtained. Based on these biophysical data, the initial nucleotides sequence was modified to increase the binding affinity (Kd) for *S. aureus* and validated using cytometry (FACS). This study suggests that structural modifications may improve the binding properties and stability of aptamers which significantly increase their applicability as recognition elements in biosensors.

KEYWORDS: *S. aureus*; aptamers; detection; biosensors; food security

ACKNOWLEDGEMENT: This work was supported in part by the French National Agency for Research (ANR-21-CE21-009 SIENA).

GR13

CLONING, OVEREXPRESSION AND CHARACTERIZATION OF A THERMOSTABLE ENDO-1,4-BETA-XYLANASE FROM *ANOXYBACILLUS VRANJENSIS* ST4

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This research deals with the characterization of a thermostable endo-1,4-beta-xylanase enzyme from *Anoxybacillus vranjensis* ST4, a thermophilic bacterium isolated from Vranjska Banja hot spring, Serbia. The enzyme shows a high degree of identity with the same type of enzyme from other species of the genera *Anoxybacillus* (97%), *Geobacillus* (74%) *and Paenibacillus* (65%). The gene for endo-1,4-beta-xylanase from the thermophilic strain ST4 was cloned into the pQE_Ek expression vector and successfully expressed and purified from the *Escherichia coli* M15[pREP4]. The study encompasses recombinant production, purification, and the comprehensive characterization of the enzymatic properties of endo-1,4-beta-xylanase. This is the first successful overexpression, purification and characterization of a recombinant thermostable endo-1,4-beta-xylanase enzyme from *Anoxybacillus*. With a monomeric structure of 38.7 kDa, the enzyme demonstrates peak activity at 70°C and pH 6.5. Notably, it exhibits remarkable stability across a wide pH range and at high temperatures, rendering it suitable for diverse industrial applications. Investigation into the enzyme's kinetic parameters, substrate specificity, and its ability to degrade xylan into high-energy value products further enhances understanding of its biotechnological potential. These findings underscore the significance of thermophilic bacteria and their thermostable enzymes in various industrial processes.

KEYWORDS: endo-1,4-beta-xylanase; microbial biotechnology; recombinant production

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GR14

THE TOXIC EFFECT OF HEAVY METAL ON *B. INTERMEDIA* AND *B. RHIZOSPHERAE* AND ITS RELATION WITH THE SEASONAL ENVIRONMENTAL FACTORS

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This study employed a systematic review technique to identify and evaluate the evidence and knowledge gaps in published studies that investigated about brucellosis in various food-producing animals in Mosul, Iraq. A variety of techniques for maintaining genetic diversity have been documented for mammalian bacterial pathogens with small genomes This investigation aimed to gain knowledge of the presence of *Brucella* spp. in the animal's soil and in sheep's blood. The first part of the collection of animal soil samples for various villages from February to November 2021 in Babylon Province, Hilla City, Iraq. The second portion of the collection was from sheep's blood samples at the end of May 2021. The soil and blood samples were cultured on *Brucella* agar and identified *Brucella* spp. by conventional PCR. After sending the positive bacterial samples isolated from the soil and blood for sequencing analysis, it was found that the bacterial samples isolated from the soil recorded in Genbank six strains for *Brucella melitensis*. Whereas deposited the two strains in Genbank for *B. melitensis* from blood. In addition, phylogenetic analysis was for the drawing of a phylogenetic tree and the determination of phylogenetic relationships. Utilization 10 ng of genomic DNA extracted from each strain to amplify this gene. Sanger sequences have been created. Isolated 16S rRNA sequences with a genus and species name (Isolated named- strains 16S aligned. fasta) were obtained from the Greengenes database. Through our research, we concluded that types of *Brucella* had genetic variations and concluded that during phylogenetic analysis of the 16S rRNA sequences, *B. melitensis* was nearest to Greece in blood samples, whereas the two strains of *B. melitensis* melitensis was nearest to India and China and the other was nearest to Mexico and USA.

KEYWORDS: *Brucella*; *B.melitensis;* soil; blood; Iraq

PP15

ACETIC ACID BACTERIA-DERIVED BACTERIAL NANOCELLULOSE: SUSTAINABLE SYNTHESIS AND ANTIMICROBIAL POTENTIAL DEVELOPMENT

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Acetic acid bacteria (AAB) are renowned for their proficiency in bacterial cellulose (BC) production, particularly species within the *Acetobacter* and *Komagataeibacter* genera. BC, derived solely from bacterial cells, represents the purest form of cellulose known, with AAB capable of generating quantities viable for commercial utilization. The utilization of bacteria for biopolymer or cellulose hydrogel production, as opposed to traditional plant sources requiring aggressive chemical treatments, presents a sustainable approach with environmental preservation benefits. While BC materials inherently lack functional properties, their porous structure and three-dimensional nanofiber network with high specific surface area render them ideal carriers for antimicrobials or other agents in the production of functional composite materials. Moreover, by-products or wastes that contain carbon or nitrogen sources for AAB could potentially substitute some part of conventional substrates, reducing production costs of BC and conserving resources. This study involved the identification of eight isolates from two kombucha beverages using molecular method, followed by screening for the most proficient cellulose-producing species. Morphological characterization of cellulose was conducted using scanning electron microscopy (SEM), while X-ray diffraction (XRD) was employed for crystallinity and phase analysis. Functionalization of nanocellulose involved the incorporation of titanium dioxide and hydroxyapatite, with elemental composition analyzed using energy-dispersive X-ray (EDS) techniques. Antimicrobial properties were evaluated using plate count tests. Additionally, discarded ethanol waste from the functionalization step was investigated for sustainable BC cellulose production. Isolated species demonstrated the production of pure, nanosized, densely intertwined BC polymers with high crystallinity. EDS analysis confirmed the presence of only carbon and oxygen elements in pure cellulose. While pure BC exhibited no antimicrobial activity, functionalized BC demonstrated antifungal and antibacterial properties. Furthermore, discarded ethanol waste proved effective for BC synthesis. These findings underscore the potential applications of functionalized BC in touch surfaces, coatings, packaging, agriculture, and medicine.

KEYWORDS: bacterial cellulose; acetic acid bacteria; antimicrobial activity; functional material

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PP16

CARBON AND GOLD APTASENSORS FOR THE DETECTION OF *ESCHERICHIA COLI*

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Fundamental for life, water has to be continuously monitored before human consumption. One of the most diffuse pathogens that can be found in water is *Escherichia coli*, which in 2022 caused 7117 human cases of Shiga toxin-producing *E. coli* (STEC) infections in Europe. As the classical microbiological analysis can require up to 5-7 days to provide a response related to the presence of *E. coli* contamination in a sample, it is obvious the need for a specific rapid method able to give the results in a short time. Thus, the development of electrochemical biosensors for the detection of microbiological contaminants in water is important. More specifically, aptasensors are biosensors built by using specific aptamers (single-stranded DNA sequences) as biological elements. In this study, an aptamer designed in silico is used to detect *E. coli* cells in water samples, and the performances of gold and carbon screen-printed electrodes are compared. Different concentrations of *E. coli* cells were tested to optimize the aptasensor. The results show a high sensibility of the new tools, with the possibility to detect a concentration of 10^1 CFU/ml of *E. coli* cells in a water sample. By testing the aptasensor with negative controls (*Enterococcus faecium* and *Salmonella*), a good specificity is reached too. According to the results obtained for the specificity and sensibility, the short time required, and the portability of the equipment, biosensors appear to be a promising technique that can be directly used for in-field analysis in the detection of pathogens in liquid matrixes.

KEYWORDS: biosensor; aptasensor; *E. coli*; rapid method

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PP17

UNREVEALING THE ANTIMICROBIAL BLUE LED LIGHT CAPACITY FOR *E. COLI* AND *L. MONOCYTOGENES* INACTIVATION

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Antimicrobial Blue Light (aBL) has been recently discovered as a non-thermic method for sanitizing food-related environments. This technology is based on the combined action of Blue Light (400-480 nm), oxygen, and endogenous or exogenous *Photosensitizers* (PS). These factors lead to the formation of Reactive Oxygen Species (ROS), which are responsible for microbial death. aBL efficacy has been elucidated against several microbial pathogens and its microbial inactivation capacity depends on Light Dose (D), wavelength, and microbial species. Thus, this study aimed to exploit the antimicrobial activity of Blue LED Lights at 405, 420 and 450 nm, against *E. coli* and *L. monocytogenes*, exploiting endogenous PS. The analysis was divided into two parts. Firstly, the microbial inactivation was assessed directly on agar plates; and secondly, tests were done on a liquid substate. The direct assessment of cells in agar plates evidenced that a D > 300 J cm^{-2} was able to inactivate the tested microbes (ca. -6.00/-7.00 log CFU/mL) at all the wavelengths in 60 min, with only the exception of *E. coli* at 450 nm. Decreasing the D, results were different based on the tested microbes and the wavelength used. For the assessment on liquid media, a D < 300 J cm^{-2} showed different behaviour of *E. coli* and *L. monocytogenes* at the three wavelengths in 120 min. During the experiments, temperature was monitored every 30 min. The results of this research highlighted that aBL is a powerful technology for microbial inactivation in food-related environments; however, the inactivation efficiency depends on the wavelength, the microbes, and the light D.

KEYWORDS: blue light; microbial decontamination; food pathogens; ROS; photosensitizer

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PP18

INFLUENCE OF CULTIVATION METHOD ON *M. BOVIS* PASTEUR 1173P2 FATTY ACID AND PROTEIN CONTENT – PRELIMINARY RESULTS

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BCG vaccine is one of the most widely used vaccines, effectively preventing tuberculosis (TB) in infants. Since the 70s *Mycobacterium bovis* BCG str, Pasteur 1173P2 has been used to vaccinate newborns in Serbia. In this study, we present fatty acid (FA) analysis and protein content of differently cultivated *M. bovis* 1173P2. *M. bovis* was cultivated in two growth media, the Sauton liquid media pellicle growth method and cultivation in Middlebrook media supplemented with OADC. FA was extracted using chloroform/methanol (2:1 v/v) and after direct trans-esterification FAs were analyzed using gas chromatography. Proteins were extracted by homogenizing the bacterial pellet in 1% sodium dodecyl sulfate/protease inhibitors, followed by centrifugation and methanol-chloroform precipitation. Proteomics analysis was performed by in-gel trypsin digestion followed by label-free relative quantification on a nano LC-ESI-MS/MS system. Cultivation in Sauton media gave higher levels of FA 14:0, 18:0, 18:1n-9, 18:3n-6 and 20:3; higher peroxidability index and lower levels of 15:0, 16:1, 17:0, 19:0, 18:2 compared to Middlebrook OADC. A total of 335 proteins were identified which showed >60% reduced expression in Middlebrook OADC media (including probable fatty acid synthase, a mycocerosic acid synthase and a putative thiosulfate sulfurtransferase), and 199 proteins with >60% increased expression (including probable oxidoreductase, a double hotdog hydratase and probable short-chain dehydrogenase). Numerous differences in cell constituents upon cultivation of *M. bovis* in two different liquid media infer that transfer from the classical cultivation in Sauton liquid media to fermentation production in Middlebrook OADC would require immunogenicity testing and/or dose adjustment.

KEYWORDS: *M. bovis* BCG Pasteur 1173P2; sauton medium; middlebrook OADC medium; fatty acid content; protein content

PP19

MORPHOLOGICAL CHANGES OF *STAPHYLOCOCCUS AUREUS* AND *SALMONELLA* ENTERITIDIS UPON EXPOSURE TO THE EXTRACT OBTAINED FROM *PLEUROTUS OSTREATUS* MUSHROOM

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In last decades, bacterial resistance to first choice antibiotics has been drastically increasing, therefore, the research of new antimicrobial substances is of great importance. This rising problem with bacterial resistance to existing antibiotics affects not only the health care institutes but also food plants. *S. aureus* and *S. enteritidis* pose a number of challenges to the food industry and cause foodborne illness in humans. In addition, due to their favourable elemental composition, oyster mushrooms (*Pleurotus* sp.) are a reservoir of bioactive compounds that give them remarkable antibacterial potential. *P. ostreatus* is of great economic importance and is the second most cultivated edible mushroom, therefore information about its possible targets on bacterial cells is of great importance for use as a dietary supplement or medicinal purposes. The results of the antibacterial assay showed that tested bacterial strains were susceptible to the methanol extract of *P. ostreatus* (PoME), while microbicidal activity was only detected against Gram-positive bacteria. Scanning electron microscopy (SEM) micrographs suggested that extract acted on cytoplasmic membrane of *S. aureus*, while the cell envelope of *S.* Enteritidis was the most likely target. Natural extracts may outperform individual bioactive compounds due to the synergistic interaction between the metabolites, which can enhance the effects of the individual components. Extracts rich in antibacterials are emerging as alternatives to synthetic antibiotics in the food and health sectors. Among these, crude mushroom extracts are particularly sought after for their diverse bioactive ingredients, as they can combat resistant strains of bacteria due to their different targets and modes of action. PoME can be used as an effective antimicrobial agent, suitable for applications aiming to eradicate foodborne pathogens, thus enhancing food safety.

KEYWORDS: *Pleurotus ostreatus;* oyster mushroom; natural preservatives; antibacterial activity; foodborne pathogens

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PP20

THE ANTI-INFLAMMATORY EFFECT OF *LIMOSILACTOBACILLUS REUTERI* B2 ADMINISTRATION

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Limosilactobacillus reuteri demonstrates a significant role in treating gastrointestinal diseases through the synthesis of various health-promoting factors. These include mucus-binding proteins, reactive oxygen species-scavenging enzymes, antimicrobial agents (reuterin is capable of inhibiting the growth of a wide spectrum of microorganisms), vitamins (folate and vitamin B12), and unique exopolysaccharides. Different strains of *L. reuteri* exhibit strain-specific anti-inflammatory effects, influencing the expression of immune-related factors such as IL-10 and TNF-α (PMID: 20798357; PMID: 22207578). Furthermore, the mitigating impact of *L. reuteri* strains on inflammation is confirmed in vivo and in vitro with the implication of an interaction between probiotics and immune cells in the intestinal mucosa (PMID: 22207578). Our study aimed to investigate the potential anti-inflammatory effects of daily treatment with autochthonous probiotic strain *L. reuteri B2* (PMID: 33932415) could have an anti-inflammatory effect on local immune response. In a 14-day experiment with Intor Swiss: Albino mice (n=10), those treated with *L. reuteri B2* ($5x10^6$ CFU/mL, 100 µl) showed a favorable impact on the gut's inflammatory environment. Histological analyses of colon samples and intraperitoneal macrophages revealed lower myeloperoxidase (MPO) activity, reduced production of superoxide ions, IFNγ, IL-6, and TNFα, along with an enhanced production of IL-10 in *L. reuteri* B2 treated mice compared to untreated ones. Notably, histopathological preparations did not show significant differences between the groups. The study suggests that *L. reuteri* B2 may be valuable for further evaluation in managing, preventing, and treating inflammatory bowel diseases. The presented findings contribute to understanding the specific anti-inflammatory effects of this strain on the local immune response, supporting its potential as a therapeutic agent.

KEYWORDS: *Limosilactobacillus reuteri;* anti-inflammatory effect; mouse model

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PP21

STAPHYLOCOCCUS AUREUS CELL ENVELOPE FITNESS IN SERUM AND MILK

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Staphylococcus aureus is a major opportunistic pathogen in mammals that causes an array of infections in humans and animals. *S. aureus* has a capacity to sense the environmental cues, adapt and express a large number of virulence determinants, including secreted and envelop associated molecules. Although several specific signaling pathways have been described in *S. aureus* fitting, the mechanisms by which a major component of its membrane, phospholipid, enable bacterium to thrive in inconsistent environments are still barely known. We sought to determine how two model environments, adult bovine serum and row cow milk, affect *S. aureus* cell pigmentation, membrane fluidity, envelop thickness, and fatty acid composition. Moreover, we tested how these host environments modify *S. aureus* resistance to oxidative stress and bactericidal action of some antibiotics. Finally, by studying bacterial caused killing in an insect infection model, a correlation between bacterial envelop fitness and its virulence was investigated *in vivo*. *S. aureus* USA300-JE2 strain predominantly synthetized *ai*15:0 branched-chain atty acid (FA), and a mixture of straight-chain FAs (mainly C18:0, C20:0). However, C14:0 and C16:0 were the shortest abounded FA incorporated from milk and serum, respectively. Interestingly, long-chain unsaturated fatty acids C18:1, C18:2, C18:3, C20:3 and C20:4 were also efficiently incorporated although *S. aureus* cannot synthetize them. Besides, milk-adapted cells were highly pigmented, had increased membrane rigidity, ticker envelop, lower envelop permeability and autolyze rate compared to serum-adapted cells. The milk-adapted cells due to the increased staphyloxanthin expression and ticker envelop survived better than the serum-adapted cells when exposed to ROS or some antibiotics. Finally, milk-adapted bacterial cells had increased virulence *in vivo.* Overall, this study unveils adaptation of *S. aureus* to fatty-acid reach host environment, underlying possible antimicrobial resistance mechanism associated with exogenous fatty-acid integration.

KEYWORDS: *S. aureus;* fatty acids; bacterial membrane; antimicrobial adaptation

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PP22

LACTIC ACID BACTERIA FROM FRESH VEGETABLES: ISOLATION AND SELECTION FOR THEIR POTENTIAL USE AS STARTERS FOR THE FERMENTATION OF PLANT-BASED MILK ALTERNATIVES

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In recent years, the consumption of plant-based milk alternatives (PBMA) derived from nuts, grains, legumes and seeds has increased due to their positive effects, including animal welfare, environmental sustainability and personal health. The aim of this study was to isolate and identify lactic acid bacteria (LAB) from vegetables that are capable of fermenting PBMA and to investigate their resistance to antibiotics. Seven samples of vegetables from Serbia were taken. Each sample was homogenized with LM17c (M17 with 0.5% [w/v] lactose) broth with cyclohexamide (0.5 mg/mL), and after overnight accumulation, all samples were incubated at LM17c and incubated at 30 °C for 48 h. All isolates were incubated overnight at 30 °C in LM17 broth and then inoculated in 5 mL of five commercially available PBMA (soy, almond, rice, cashew and coconut). After overnight incubation at 30 °C, each fermented PBMA was visually analyzed and the pH measured. The isolates that fermented each of the five commercially available PBMA were selected for further analysis. The total DNA of all LAB isolates was extracted using the phenol-chloform extraction method. For rep-PCR analysis, the total DNA of the different LAB isolates was used as a template for PCR amplifications with Random Amplified Polymorphic DNA (RAPD). Antibiotic resistance of selected isolates was determined using the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute. The results show that 115 out of 143 isolates ferment each of the five commercially available PBMA. The measured pH of PBMA after fermentation decreased compared to the control PBMA (PBMA without isolates). The results of the rep-PCR analysis revealed 35 different isolates. Antibiotic resistance tests showed that all 35 strains were sensitive to penicillin, tetracycline, chloramphenicol, erytromycin and ampicillin. In addition, most strains were sensitive to vancomycin and all strains were resistant to streptomycin.

KEYWORDS: plant – based fermentation; lactic acid bacteria; antibiotic resistance

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PP23

INFLUENCE OF CLOVE ESSENTIAL OIL ON THE GROWTH OF FERMENTATIVE YEASTS

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Cloves are the aromatic, dried flower buds of the tree *Syzygium aromaticum, which are* often used as a spice to flavor fermented beverages and as a preservative against oxidative rancidity in food rich in fat. In addition, cloves are traditionally used as a natural remedy to treat various ailments such as gastric irritability, flatulence colic, chronic diarrhea and other gastrointestinal disorders. The biological activities of this spice, such as antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, analgesic, insecticidal, and many others, are due to a mixture of different compounds, among which the three main active compounds eugenol, eugenol acetate and *β*-caryophyllene are stand out. The aim of this study was to evaluate the influence of commercial clove essential oil on the growth of fermentative yeasts from *Saccharomyces* and non-*Saccharomyces* groups and to assess its use in the production of fermented beverages such as wine in place of sulfites as a preservative. The antifungal efficacy was investigated by agar well diffusion, agar dilution and broth *macrodilution* methods, and the results obtained in this study were compared. According to the results of the antifungal activity of clove essential oil, the most sensitive yeast was *Kloeckera* ssp. from the non-*Saccharomyces* group with a minimum inhibitory concentration (MIC) of 0.02% (v/v) and a minimum fungicidal concentration (MFC) of 0.1% (v/v). From the *Saccharomyces* group, the lowest value for MIC and MFC was determined for *Saccharomyces boulardii* (MIC 0.55% (v/v), MFC 0.08% (v/v), while the other species from this group were slightly more resistant in the following order *Saccharomyces cerevisiae*>*Saccharomyces cerevisiae* var. *diastaticus*>*Saccharomyces pastorianus*> *Saccharomyces cerevisiae* var. *ellipsoideus.* Due to its antioxidant and antimicrobial properties, clove essential oil has the potential to be used as a substitute for sulfites in controlling the growth of surface yeast populations on fruit during spontaneous fermentation and the production of fermented beverages such as wine.

KEYWORDS: fermentative yeasts; clove; essential oil; eugenol; antifungal activity

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PP24

GLYCOSIDE HYDROLASES FROM FRESHWATER FISH GILL MICROBIOTA AS BIOFILM INHIBITORS FOR ENHANCED FOOD SAFETY

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The formation of biofilms by foodborne pathogens is a constant challenge in the food industry, leading to an increased risk of contamination and compromising food safety. Many of the chemicals commonly used for sanitation in the food industry are unable to remove biofilms, are harmful to surfaces and can be toxic. The effectiveness of disinfectants can be improved using enzymes that specifically target biofilm components such as exopolysaccharides, extracellular DNA, or proteins. In this study we investigated the potential of glycoside hydrolases originating from the gill microbiota of freshwater fish to control biofilm formation in the most common foodborne pathogens. We demonstrated that *β*-glucosidase from *Microbacterium* sp. BG28 (BglB-BG28) effectively inhibits cellulose-rich biofilms formed by *Salmonella enteritidis*, *S. typhimurium*, *S. infantis*, and *Escherichia coli*. When these bacteria were cultivated overnight with 200 µL/mL enzyme, up to 80% less biofilm was formed. By fluorescence microscopy, we visualised the inhibition of biofilms on plastic, glass and aluminium, materials commonly used in the food industry. When used as a pre-treatment, BglB-BG28 increased the bactericidal efficacy of Oxicid®S, a commercially available surface disinfectant. Its effectiveness at temperatures up to 50 °C and in a pH range from 4 to 8 together with compatibility with non-ionic detergents and high tolerance to sodium chloride and glucose give BglB-BG28 advantages in harsh and diverse industrial environments. Importantly, no toxicity to *Caenorhabditis elegans* was observed at enzyme concentrations of up to 1 mg/ml. Overall, these results demonstrate the suitability of the *β*-glucosidase BglB-BG28 for the formulation of a novel enzyme-based disinfectant to be used in food processing facilities.

KEYWORDS: biofilm; foodborne pathogens; enzyme; *Salmonella*; *Escherichia*

ACKNOWLEDGEMENT: This study has been funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (grant number 451-03-47/2023-01/200042).

PP25

LEUCONOSTOC MESENTEROIDES DSA_PM01A5 PRODUCES A MIXTURE OF DEXTRAN AND LEVAN WITH ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES

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Over the last few decades, exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) have gained the attention of researchers. This is due to their well-known technological properties, which include thickening agents, water retention agents, and emulsifiers. Additionally, they possess bioactivities such as antimicrobial, antioxidant, antibiofilm, and prebiotic properties. However, each EPS has unique structural features that determine their properties, such as molecular weight, presence of branches, and functional substituent groups. Furthermore, the chemical features of EPS are closely related to the producing species and strain, and each microbial strain can produce unique EPS with peculiar chemical, technological, and bioactive features. Thus, the continuous isolation of LAB capable of producing EPS and the characterization of the polymers is of great interest. A study was conducted to structurally characterize and check the bioactivities of an EPS produced by *Leuconostoc mesenteroides* strain DSA_PM01A5 isolated from sourdough. The EPS was a mixture of dextran and levan, which was characterized chemically through NMR, FT-IR, and HPLC. The study found that the EPS hindered the growth of selected foodborne pathogens (*Escherichia coli*, *Salmonella enterica*, *Enterococcus faecium*, and *Listeria monocytogenes*) and inhibited the biofilm formation by *Listeria monocytogenes* and *Pseudomonas fluorescens*. Additionally, the EPS demonstrated good antioxidant activity in terms of DPPH radical inhibition, suggesting its possible use as an antioxidant agent in foods. Moreover, the EPS provided mild protection from oxidative DNA damage, which may be a good starting point for any *in vivo* test. While all these features could lay the foundation for the possible use of EPS as antimicrobial and antioxidant agents, further investigation of the mechanisms underlying the observed bioactivities is necessary.

KEYWORDS: exopolysaccharides; *Leuconostoc;* dextran; levan; antimicrobial activity; antioxidant activity

PP26

EXPLORING THE BIOTECHNOLOGICAL POTENTIAL OF THERMOPHILIC BACTERIA - DERIVED PECTIN LYASES: A MINI-REVIEW

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Bacteria are an ideal source for producing pectin lyases (PNLs) due to their amenability to laboratory cultivation and genetic manipulation, which facilitates enhanced enzyme production. Predominantly originating from various thermophilic bacteria, bacterial PNLs usually exhibit alkaline properties, although cases of acidic variants have also been documented. In particular, a thermostable alkaline pectin lyase, displaying optimal activity at 60°C, has been characterized from the thermophilic bacterium *Brevibacillus borstelensis* P35. Similarly, thermostable acidic PNLs have been identified in *Geobacillus stearothermophilus* Ah22 and *Bacillus subtilis* SAV-21. Thermophilic bacterial species are emerging as significant and highly efficient sources, boasting diverse enzymatic repertoires, including pectinolytic enzymes, rendering them attractive candidates for various biotechnological applications. This mini-review focuses on the characterization of pectin lyases from a thermophilic bacterium, shedding light on its biochemical properties, substrate specificity, and potential industrial applications. Enzymes exhibit outstanding biochemical properties, with optimal pH and temperature ranges conducive to industrial processes, along with notable thermostability and pH tolerance, augmenting their suitability for diverse biotechnological endeavours. Furthermore, the enzyme demonstrates specificity towards pectin, efficiently cleaving glycosidic bonds within the polysaccharide backbone. Understanding the substrate specificity of pectin lyases is crucial for its effective utilization in industrial processes, especially considering its preferences for high-methoxylated pectin while still demonstrating activity on low-methoxylated and amidated pectins, expanding its applicability. Additionally, the synergy of pectin lyases with other pectinolytic enzymes enhances the efficiency of pectin degradation, facilitating the production of valuable products such as biofuels, dietary fibers, and oligosaccharides. The versatility and efficiency of pectin lyases from thermophilic bacteria highlight its potential for application across various biotechnological sectors, including food and beverage, textile, and pharmaceutical industries. Its capability to modify pectinaceous materials offers sustainable solutions for waste valorization and bioconversion processes.

KEYWORDS: thermophilic bacteria; pectin lyases; thermostable pectin lyase

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PP27

CAMPYLOBACTER SPP. CLASSICAL AND RAPID DETECTION

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Campylobacter spp. is responsible for gastroenteritis and severe diseases. Insufficiently cooked poultry products are identified as the major source of *Campylobacter* spp., suitable methods for routine meat analysis are required. The official culture-based methods take almost 5-7 days, a long time for poultry-based products usually consumed in few days. Last years, biosensors have got high interest for routine analysis as they are specific, rapid, simple to use, and cheap compared to other methods. Studies have been conducted to improve sensitivity and specificity for *Campylobacter* detection in food samples, from enrichment medium to molecular methods which can reduce time, but all these methods require the delivery of samples to equipped laboratories with trained personnel. Food industry needs rapid and sensitive methods for the detection of this pathogen. Biosensors can respond to these requirements, moreover they can be useful for in field tests. Poultry samples have been tested by using the ISO 10272-1:2006 method, the qPCR protocol based on CampyPFw and CampyPRv primers and an electrochemical genosensor. CampyPFw and CampyPRv primers showed specificity and sensitivity towards *Campylobacter* species when used in qPCR to examine chicken meat samples reaching detection levels below 10^3 CFU/mL. The qPCR results confirmed the ISO 10272-1:2006 method results. The biosensor tests were performed with the CampyP3 DNA probe (patent SIB BI803E/RVP/rmc, 2020, it 102020000012496) have reached the sensitivity about 2 pg/µL. The presence of *Campylobacter* in chicken meat samples was confirmed by all methods, the main differences detected were about the required time for results, ease and sensitivity. Moreover, the limit of detection showed by the genosensor can be improved increasing the concentration of the DNA probe. This electrochemical genosensor can be an alternative to classical analyses, moreover, has a great potential as a point-of-care device for analyses of food samples for detection of *Campylobacter* in foods.

KEYWORDS: *Campylobacter* spp.; electrochemical genosensor; poultry meat; rapid method

PP28

TRICHODERMA DETECTION IN MUSHROOM SUBSTRATES: UTILIZING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY WITH GOLD NANOPARTICLES FOR COLORIMETRIC ANALYSIS

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One of the significant challenges in organic cultivation of edible mushrooms is the control of invasive *Trichoderma* strains, which can hinder mushroom production and lead to significant economic losses. The development of tools for early and specific detection of these pathogenic fungi is of great importance. This study aimed to develop new method for fast detection of *Trichoderma* using a loop-mediated isothermal amplification (LAMP) assay combined with gold nanoparticles (AuNPs). The assay relies on preventing salt-induced aggregation of AuNPs through amplicons generated in positive LAMP reactions. The observable color change from red to violet with the naked eye facilitates easy operation, making the LAMP-AuNPs assay a simple initial screening tool for rapid Trichoderma detection in mushroom cultivation substrate. The AuNPs were synthesized using the reduced citrate method. LAMP amplification of gDNA templates was conducted at 65°C for 30 minutes. Assay specificity was determined using *T. harzianum* gDNA as a positive control, and negative LAMP reactions were performed without a DNA template or with non-specific gDNA from *B. subtilis* BSB1. Assay sensitivity was estimated by involving a 10-fold serial dilution of positive LAMP reaction products in the context of *T. harzianum* gDNA detection. The developed LAMP-AuNP assay has demonstrated its effectiveness as a rapid and specific method, with a detection limit of 24 ng/µL for LAMP amplicons, discernible by the naked eye. This assay represents a promising alternative to other analytical methods integrated with LAMP and introduces an innovative approach to *Trichoderma* detection.

KEYWORDS: *Trichoderma* spp.; LAMP; AuNPs; detection; mushroom cultivation

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PP29

ANTIBACTERIAL ACTIVITIES OF ETHANOL AND ACETONE EXTRACTS OF *AGARICUS BISPORUS*

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Button mushrooms (*Agaricus bisporus*) are one of the most widely consumed nutritionally rich mushrooms. The presence of terpenes, phenolic and flavonoid compounds and glycoproteins indicate that mushrooms can exhibit many therapeutic properties. Here we examined whether extracts from *A. bisporus* possess the antibacterial activity (ABA), by investigating its chemical composition and biological activity. The ABA of ethanol (E) and acetone (A) extracts of white (W) and brown (B) button mushrooms was tested using the agar well diffusion method and high-performance thin-layer chromatography-direct bioautography (HPTLC-DB) assays on four bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), and *Escherichia coli*). HPTLC fingerprint derivatized with anisaldehyde reagent solution was done to detect compounds present in the extracts. All four extracts showed similar HPTLC profile with more pronounced bands at *h*RF > 70 in AW and AB extracts. Active bands representing compounds with ABA at HPTLC-DB biochromatograms were more pronounced in acetone extracts. In all four biochromatograms, dominant bands exhibiting ABA are located at *h*RF 86 and 94. The biochromatograms developed for *S. aureus* and MRSA have active bands of lower intensity, while the *E. coli* biochromatogram is very similar to the biochromatogram for *B. subtilis*. Principal component analysis (PCA) was performed to compare ABA of the extracts and their grouping. Gram-positive biochromatograms' objects were separated from the *E. coli* biochromatogram's objects. However, the objects of Gram-positive biochromatograms are not uniform, but they are separated from each other. The agar well diffusion method confirmed the ABA of the whole content of extracts. All four extracts exert ABA forming inhibition zones on all tested bacteria. This study suggests that button mushrooms possess the antibacterial activity towards different bacterial strains. The identification and validation of individual active compounds separated on HPTLC-DB biochromatograms is under progress.

KEYWORDS: antibacterial activity (ABA); button mushrooms; *Agaricus bisporus*; high-performance thin-layer chromatography-direct bioautography (HPTLC-DB); agar well diffusion method

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PP30

LACTOBACILLUS SP. STRAINS FROM SERBIAN TRADITIONAL CHEESES: CHARACTERIZATION, ANTIMICROBIAL ACTIVITY AND ANTIBIOTIC RESISTANCE

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Traditional cheeses are ideal matrices for diverse microorganisms due to artisanal production methods. Some strains within such products, particularly certain *Lactobacillus* species, have demonstrated their application in biotechnology as protective culture or probiotic bacteria, offering both unique flavours and potential health benefits. The aim of the study is to isolate and characterize *Lactobacillus* strains sourced from a diverse array of Serbian traditional cheeses. Subsequently, the selected *Lactobacillus* isolates are subjected to examination of their antibiotic susceptibility to ten antibiotics, as well as their antimicrobial activity on ten pathogenic microorganisms to determine their potential in inhibiting growth. The sampled cheeses are traditionally produced using goat's or cow's milk from eleven locations (Čačak, Trstenik, Donja Bukovica, etc.). From 112 tested isolates, 57 were Gram-positive and catalase-negative. Two isolates grew at 15°C and 24 at 45°C. All tested isolates showed homofermentative metabolism. Further analysis revealed that 36 isolates displayed growth in broths containing 4% NaCl, 5 isolates thrived in broths with 6.5% NaCl and none of the isolates exhibited growth in broths of 8% NaCl. Molecular characterization using the RAPD method narrowed down the pool to 34 isolates. After subjecting the samples to PCR testing, signals on the gel indicated that 30 out of the 34 isolates belonged to the genus *Lactobacillus*. Out of 30 isolates tested for sensitivity to antibiotics, two isolates were resistant to ampicillin, while one isolate was resistant to chloramphenicol. All isolates effectively inhibited *Salmonella enteritidis* and *Yersinia enterocolitica*, while two strains isolated from Ćuprija goat cheese showed the most success in inhibiting the growth of 9/10 pathogenic microorganisms. None of the strains showed antimicrobial activity against *Candida albicans*. This study confirms earlier results of the application of *Lactobacillus* strains with antimicrobial activity or potential probiotics isolated from traditional dairy products.

KEYWORDS: *Lactobacillus*; traditional cheeses; antimicrobial activity; antibiotic susceptibility

PP31

RESEARCH AND APPLICATION OF MICROBIOLOGICALLY SYNTHESIZED POLYSACCHARIDES AS POTENTIAL PREBIOTICS

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Prebiotics are nondigestible ingredients of food, which selectively stimulate the growth and metabolic activities of health-promoting bacteria in the intestinal tract, and have beneficial effects on health. They have been widely used in human and animal nutrition since have positive biological activities. The main source of prebiotics is plants, however, microorganisms can produce polysaccharides which could have prebiotic properties. Microbial polysaccharides can be applied in nutraceutical delivery since they can form gelled matrices or nanoparticles. This study aimed to test *in vitro* the microbiologically synthesized polysaccharides levan, pullulan, and *β*-glucan. This is the first step in analyzing their potential as prebiotics. Levan was isolated from *Bacillus licheniformis* NS032 strain, pullulan from *Aureobasidiumpullulans* CH-1 and *β*-glucan from baker's yeast *Saccharomyces cerevisiae*. Prebiotic properties of these microbial polysaccharides were studied on the growth of standard strains of *Lactobacillus* and *Bifidobacterium*, as well as on bacteria isolated from infants' feces. The determination of biochemical parameters and gas production was further criteria for the selection of potential prebiotics. Also, the forming of film coupled with their unique physicochemical properties has been researched.Results showed positive effects of investigated polysaccharides on the growth of individual strains or a consortium of microorganisms isolated from the infant's feces. Investigated polysaccharides also show a tendency to form films, which can be used in food. According to the results, polysaccharides showed different effects of potential prebiotic on selected individual probiotics or consortiums, which can be further tested *in vitro* and *in vivo* for application in nutrition and supplementation.

KEYWORDS: prebiotics; levan, pullulan; *β*-glucan

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PP32

ISOLATION AND IDENTIFICATION OF *LEGIONELLA* SPP. IN WATER SAMPLES

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Legionella is a ubiquitous microorganism that causes Legionnaires' disease. These Gram-negative bacilli have specific nutritional requirements and are most often found in water systems and are relatively resistant to the effects of heat and chlorine. The most common and most virulent species associated with human infections is *Legionella pneumophila* serogroup 1. Other serotypes of *L. pneumophila*, as well as other species of *Legionella sp*., may be less frequently associated with human disease, especially in immunocompromised individuals. Isolation and identification of *Legionella* sp. in water samples was performed according to SRPS EN ISO 11731:2017 with confirmation of identification by the Maldi tof Vitek MS method. 967 water samples of different origins were examined, among which the presence of *Legionella sp*. Is confirmed in 163 samples (16.85%). The most common isolate was *L. pneumophila* serogroup 1, 133 (81,6%), followed by *L. pneumophila* serogroup 2-14, 30 (18,4%). Tested water samples in which the presence of *Legionella* sp. were mostly warm water samples with temperature values of 40-59°C (>90%), water samples that generate aerosols, while chlorine preparations did not affect its presence. The largest number of *Legionella pneumophila* sg 1 positive samples were hot water samples from hotel and spa showers. *Legionella pneumophila* sg 2-14, was isolated in water samples from the hospital, as part of an epidemiological investigation due to the illness of newborns. The previous monitoring of *Legionella* sp. shows the significant presence of this bacterium in various water samples, both in hotels and recreational centers, and in the hospital environment. For this reason, it is necessary to increase the scope and frequency of control of certain types of water, as well as improve the legal legislation and harmonize it with the regulations of other countries.

KEYWORDS: *Legionella pneumophila*; legionnaires' disease; water quality control

PP33

POLYPHENOL PROFILE AND ANTI-TYROSINASE POTENTIAL OF THE POLYPORE MUSHROOMS *FOMITOPSIS PINICOLA* AND *GANODERMA LUCIDUM*

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Tyrosinase is widely present in plants and mushrooms and is responsible for enzymatic browning reactions in damaged foodstuff. Accordingly, its inhibitors act as anti-browning compounds and have an important role in maintaining food quality. In the cosmetic industry, inhibitors of tyrosinase have important applications as skin-lightening agents. Due to the ability of phenols to react with proteins, the potential of polypore mushrooms methanol extracts to inhibit tyrosinase was investigated. Fresh wild-growing fruiting bodies of the two mushroom species, namely *Fomitipsis pinicola* and *Ganoderma lucidum*, were collected from the Kopaonik and Avala mountains, Republic of Serbia. Their methanol extracts were analyzed for the total phenol content (TPC) and phenolic profile using liquid chromatography coupled with mass spectrometry (LC-MS/MS). The tyrosinase inhibitory potential was determined in the reaction solution of 46 units/mL tyrosinase and 2.5 mM of dihydroxyphenylalanine (L-DOPA). Results were expressed as IC50 values, the concentration of extract required for 50% in vitro inhibition. The results showed that TPC levels were from 38.6 to 133.1 mg gallic acid equivalent (GAE) of extract dry weight (DW), with *F. pinicola* having the highest level. *F. pinicola* extract displayed the strongest tyrosinase inhibitory activity (0.10 mg/mL) almost comparable with kojic acid (0.079 mg/mL) commonly used as a standard inhibitor of tyrosinase. A very strong and significant correlation between TPC and IC50 values in tyrosinase inhibition was observed ($r = -0.96$). Gallic acid (951.12 µg/g extract DW) was found to be the main polyphenol ingredient of *F. p*inicola extract. In addition, the anti-tyrosinase activity exhibited by investigated methanol extracts could also be attributed to the presence of other phenolic acids like protocatechuic, p-hydroxybenzoic, chlorogenic, vanillic, p-coumaric, and caffeic acid. The results of the present study suggest that methanol extract of the polypore mushrooms *F. pinicola* and *G. lucidum* originating from Serbia act as natural tyrosinase inhibitors and are rich sources of phenolic acids. These mushrooms may be a good material for the development of anti-browning additives as well as additives in skin-lightening cosmeceutical formulations.

KEYWORDS: polypore mushrooms; phenolic acids; tyrosinase inhibition; anti-browning; skin-lightening

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PP34

PREDICTIVE MODEL OF FOODBORNE PATHOGENS GROWTH IN MINCED PORK

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Mathematical microbial models predict the response of microorganisms, including their growth, survival or inactivation, in food during processing and storage. Predictive models can give results faster than a traditional challenge test regarding food safety, quality, and shelf life. However, they do not replace laboratory analysis, and must be used with great caution. This study was performed to assess the survival of four foodborne pathogens, including *Salmonella enteritidis*, *S. typhimurium*, *Listeria monocytogenes*, and *Yersinia enterocolitica,* in minced pork stored at 4 °C during 96h applying the Barany and Roberts model. The maximum growth rate (μmax) ranged from 0.00075 log CFU/g/h for *S. typhimurium* to 0.016 log CFU/g/h for *Yersinia enterocolitica*. According to the coefficient of determination (R^2 0.83-0.93), and the standard error (SE 0.03-0.16) the applied Barany and Roberts model was reliable in predicting the behavior of foodborne pathogens in minced pork stored at 4 °C.

KEYWORDS: *Salmonella enteritidis*; *Salmonella typhimurium*; *Listeria monocytogenes; Yersinia enterocolitica;* Barany and Roberts model

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PP35

AEROBIC APPROACH FOR THE CONVERSION AND VALORIZATION OF BIODEGRADABLE WASTE

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The growth of the world's population, demographic change, urbanization, the development of living standards and the economic boom have accelerated the generation of enormous quantities of municipal solid waste. The urgent need to manage the waste generated, combined with the depletion of resources, has triggered the transition from a wasteful linear economic model to a sustainable circular economic model. The consequences of the linear economy range from climate change, pollution, land and resource scarcity to biodiversity loss. Society therefore needs to rethink the way it deals with waste. This way of thinking has led to waste streams being increasingly recognized as valuable. The fundamental goal of the circular bioeconomy is to valorize and reuse waste as secondary raw materials. Valorization includes any process of recycling, reusing or converting waste materials into resources. The microbial conversion of the biodegradable waste fraction into valuable compounds is a promising way to pursue the goals of the circular economy. Composting is an aerobic process of waste decomposition in which organic matter is broken down by the action of microorganisms, bringing benefits such as valorization, sterilization, stabilization and reduction of waste biomass. In this work, the composting of biodegradable municipal solid waste was carried out in a closed reactor system. Physico-chemical and microbiological analyzes were carried out during the composting process. The microbial conversion of the biodegradable municipal solid waste was around 56 %. Composting is considered a sustainable method of organic waste management. Recycling biodegradable waste into biofertilizer and soil amendment has great potential to combat soil degradation in agricultural areas.

KEYWORDS: biodegradable waste; composting; conversion; valorization

ENVIRONMENTAL MICROBIOLOGY AND BIOTECHNOLOGY

IL36

HARNESSING SPATIAL MICROBIOME DYNAMICS FOR CUTTING-EDGE ENVIRONMENTAL BIOTECHNOLOGY

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The utilization of microorganisms in diverse environmental applications, including bioremediation, agriculture, renewable energy generation, and biomass production, has garnered significant attention. Current approaches primarily involve the use of consortia isolated from the environment, bottom-up construction, or metabolic engineering. However, efficient functionality of microbial communities extends beyond the mere presence of microbes and their predicted metabolic compatibility, as exemplified by natural structures like biofilms and aggregates. The spatial distribution of various species within these structures offers a unique perspective on the assembly of bacterial communities. In this presentation, we detail our methodology for positioning cells within various structures using fundamental physical forces, specifically electromagnetism. Microbial cells typically exhibit a negative surface potential under normal physiological conditions, attributed to negative chemical groups distributed within the membrane and cell wall. Overcoming the repulsive forces generated by this strong surface charge is essential for the attachment of cells to each other. Our laboratory focuses on developing approaches based on electrostatic interactions, allowing for the physical attachment of live bacterial cells to other cells or different inanimate surfaces. Through the manipulation of conditions such as ionic strength, flow, cell density, etc., we have constructed synthetic polymicrobial structures, including biofilms and aggregates. These structures not only bring cells together but also enable spatial distribution through intentional design. Our studies on the formation and development of these structures have provided insights into the self-organization of communities, niche separations, and the rewiring of ecological interactions from competition to cooperation. This foundational knowledge has been translated into practical applications, including the precipitation of toxic metals (Zn, Pb, U) in polluted waters and the degradation of organically polluted waters and soils. Smart carriers designed through our approach have shown promise in bioaugmentation strategies for bioremediation.

KEYWORDS: biofilms; synthetic communities; microniche structuring; bioremediation; bioaugmentation

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IL37

NEW PERSPECTIVES FOR MICROBIOLOGY AND BIOTECHNOLOGY IN CULTURAL HERITAGE RESEARCH

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The established narrative surrounding microorganisms in cultural heritage is predominantly negative, primarily focusing on microbially-induced deterioration of heritage objects and potential threats to human health. As a result, research and conservation efforts are primarily aimed at eliminating microorganisms. In recent decades, new perspectives for microbiology in cultural heritage have emerged, shedding light on the complex relationships between microbes and cultural heritage objects. In our interdisciplinary study, we explored the microbial communities concealed within written heritage objects by integrating traditional microbiological cultivation and physiological studies with biomolecular analyses (such as high-throughput sequencing, genome mining, and comparative genomics), alongside historical and scholarly heritage research, to examine the microbial communities of medieval manuscripts. We successfully cultured a relatively large number of microorganisms inhabiting a medieval parchment Bible from the 14th century, uncovering the microbial diversity hiding in plain sight. We identified endospore-forming microorganisms (representing several *Bacillaceae* genera) with a significant proportion of extremophiles, such as alkaliphilic and halophilic bacteria. Their adaptation reflects the physicochemical habitat intrinsic to the materiality of the cultural heritage artifact and the medieval manuscript production techniques. Genome mining of representative novel isolates revealed the presence of genes associated with stress tolerance mechanisms (such as arsenic resistance) and adaptation to osmotic stress (such as the complete ectoine biosynthesis pathway). The genomic potential harboured in these microbial communities renders them of biotechnological interest. Furthermore, we hypothesize that some of the metabolic processes driven by such microorganisms could be harnessed to aid in the conservation of heritage objects. Our study sheds light on the microbial communities associated with cultural heritage objects, revealing novel microbial diversity thriving in environmental extremes and an untapped source of biomolecules with biotechnological interest, promoting a broader understanding of the complex interplay between microbes and cultural heritage.

KEYWORDS: cultural heritage; environmental microbiology; biotechnology; extremophiles; biodiversity

ACKNOWLEDGEMENT: This work was supported by the Federal Ministry of Education and Research (BMBF), grant #01U01811A-C: Contamination and Legibility of the World: Articulating Microbes in Collections (MIKROBIB).

IL38

USING FITNESS LANDSCAPES TO ENGINEER OPTIMAL FUNCTION IN MICROBIAL COMMUNITIES

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Microbial communities are a promising tool for the development of new sustainable solutions in agriculture and biotechnology. However, rationally engineering microbial communities remains a major challenge. The functions and services that microbial communities provide depend on an intricate network of interactions feeding back between species and their environment, making it extremely difficult to predictively link the composition of a community to its function. Because the number of possible interactions scales combinatorially with the size of the consortium, the experiments required to characterize the function of combinations of a set of species become infeasible for even moderately large communities. Alternative approaches characterize the function of larger communities by coarse-graining their composition to species diversity, thereby losing species-level resolution. To overcome these limitations, we extend the theory of fitness landscapes from quantitative genetics to map the species composition of a community to its function. Using this theory, we quantitatively dissect how species interactions at the functional level drive the collective function of the community. We then demonstrate that the complex network of interactions underlying community function often distills into simple species-by-ecosystem functional relationships, mimicking "global epistasis" patterns in genetic networks. These global epistasis patterns manifest as simple linear trends linking the functional effect of adding a species to the function of the receptor community. I show how global epistasis can be leveraged to predict the function of arbitrary communities from their species composition with minimal experimental effort. I will discuss different approaches to this end, ranging from relatively simple application of linear regressions to more sophisticated machine-learning approaches. Our work illuminates an unexplored path toward the rational engineering of ecological systems, with potential use across agricultural, biotechnological, and environmental applications.

KEYWORDS: microbial consortia; microbial community engineering; fitness landscapes; theory in microbial ecology

IL39

PHYTOPLANKON IN SMALL WATER BODIES

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Small and generally shallow freshwater ecosystems, both natural and artificial, play an extremely important role as the centers of the biodiversity preservation, as well as a source of various raw materials for the pharmaceutical and food industries. They are also the places where the water is being purified naturally, they have an impact on mitigating of climate change, etc. However, this type of ecosystem is not free from the strong negative impact of human activities, especially pollution by nutrients, organic matter and various other pollutants. The increased nutrient inflow mainly affects the increase in the biomass of primary producers. Although macrophytes are the dominant primary producers in such ecosystems, phytoplankton also plays an important role and is considered as one of the biological elements for assessing the ecological status/potential of small aquatic ecosystems. The study of the qualitative and quantitative composition of phytoplankton in various shallow water ecosystems of Vojvodina province (channels, abandoned riverbeds, fishponds, protected ponds, gravel pits) over the last ten years has shown that macrophytes have the greatest influence on the state of phytoplankton. When macrophytes are abundant and diverse, the phytoplankton abundance is under control. On the other hand, the uniformity in a structure of macrophyte community and the dominance of reeds allow an excessive development of phytoplankton and usually lead to a large number and high biomass of potentially toxic cyanobacteria. Such a situation is particularly harmful in fishponds, but also in other aquatic ecosystems used for recreational purposes, since the cyanotoxins that can be produced in that case affect the entire living world, the aquatic environment and thus ecosystem services obtained from these important habitats. Maintaining abundant and diverse macrophytes is critical for reducing the risk of cyanotoxin occurrence in small freshwater ecosystems.

KEYWORDS: ecological status/potential; macrophytes; cyanotoxins, microalgae

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IL40

SOCIAL STRATEGIES OF BENEFICIAL BACTERIA *BACILLUS SUBTILIS*

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Bacteria form diverse multicellular assemblages where they engage in intricate social dialogs that shape their survival and adaptation. However, mechanisms shaping bacterial sociality and how it affects bacteria and their hosts are still poorly understood. To bridge this gap in understanding we apply advanced confocal microscopy and genetics to investigate *Bacillus subtilis* sociality focusing on intraspecies and interspecies sociality, particularly with enteropathogenic bacteria, and also on the effects of these interactions on plant and animal hosts. Our investigations revealed that closely related *B. subtilis* isolates merge their swarms but less related conspecifics form boundary upon swarms' contact, which disclosed that *B. subtilis* species employs kin discrimination (KD) like behaviour. KD between *B. subtilis* genotypes depends on the presence/absence of genetic loci that encode toxins-immunity proteins, bacteriocins, antibiotics, stress response regulators and cell wall components. Recent results showed that KD, intensely studied in humans, animals, and plants, but less so in bacteria, significantly impacts the spatial distribution and fitness of interacting *B. subtilis* genotypes in swarms and biofilms. KD also facilitates horizontal gene transfer between non-kin swarms and serves as a policing mechanism, curtailing the spread of cheaters within the swarming populations. Moreover, our recent results aiming to investigate host responses to bacterial KD interactions revealed that plants differentially activate transcriptional responses to kin versus non-kin *B. subtilis* biofilms grown on their roots. Also, this line of experiments identified a plant gene that controls abundance of *B. subtilis* in plant tissue and consequently plant health. By uncovering the secrets of bacterial social behaviours, we move closer to applying these insights for innovative applications of beneficial bacteria in food production and sustainable agriculture.

KEYWORDS: kin discrimination; biofilm; horizontal gene transfer; plant transcriptomics; probiotics

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IL41

A CONSORTIUM OF PLANT-BENEFICIAL MICROORGANISMS MITIGATES DROUGHT EFFECTS ON MAIZE BY AIDING THE RECRUITMENT OF FOCAL SOIL MICROORGANISMS IN RHIZOSPHERE

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Utilizing the plant-microbe interactions through inoculants containing plant beneficial microorganisms (BMs) is a promising method for improving agricultural sustainability. BM inoculants can promote plant growth through various mechanisms, but their performance varies under field conditions. To gain insights on the factors that determine the performance of BM inoculation we herein tested how BM inoculation affects the plant growth and plant characteristics under field conditions and how the observed growth promotion associates with bacterial and fungal community composition. To address our research question performed two field experiments with a consortium of BMs (BMc) composed by *Bacillus atrophaeus* ABi03, *Pseudomonas* sp. RU47 and *Trichoderma harzianum* OMG16. We drench-inoculated maize plants grown under different tillage and N-fertilization intensities in a long-term field experiment located in central Germany, collected rhizosphere samples, and performed 16S rRNA gene amplicon and shotgun metagenomic sequencing. BMc inoculation increased plant biomass and iron uptake in 2020, during a prolonged drought, but not in 2021, during average precipitation conditions (based on the region's average precipitation). We identified ASVs, which positively associated with iron uptake, by using log-linear models. We cross-validated the models with random forest regression: these ASVs explained in total 50% of variance of iron uptake. Moreover, the iron-associated ASVs significantly increased due to BMc inoculation in 2020, but not in 2021. However, in 2021 these iron-associated ASVs showed a generally higher abundance than in 2020, independently of BMc inoculation. Based on random forest regression several ASVs taxonomically classified as *Comamonadaceae* were the most important predictors for iron uptake. Furthermore, we performed comparative genomics of available public data from genomes phylogenetically related to our ASVs, where we found common plant-beneficial traits. Consequently, our results indicate that BMc inoculation indirectly improved maize growth during stress exposure by aiding the recruitment of focal soil microorganisms.

KEYWORDS: plant microbiology; plant-microbe interactions; microbial ecology; modelling

IL42

COMPARATIVE ANALYSIS OF PHYLLOSPHERE MICROBIOTA IN OLIVE LEAF SPOT DISEASE

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Olive leaf spot (OLS), caused by *Venturia oleagina* (Castagne) Rossman & Crous (syn. *Spilocaea oleaginea*), is one of the most widespread and important disease of olive trees. Infected trees are weakened, characterized by severe defoliation, which impairs fruit ripening and reduces fruit set in the subsequent years. To investigate the microbial communities of infected leaves and determine differences compared to asymptomatic leaves, a metabarcoding approach targeting the ITS1 region and V4 hypervariable region of 16S rRNA was employed. Concurrently, the culturable microbiota was characterized. Morpho-anatomical analysis of symptomless and infected olive leaves was conducted to gain detailed insight into disease progression. The *Venturia* genus was confirmed as significantly more abundant in infected leaves. The alpha diversity indices (Shannon and Simpson) revealed a slightly lower diversity of mycobiota in symptomless leaves, while bacteriobiota analysis showed a significantly reduced Shannon index in infected leaves. Among the differentially abundant (DA) genera, *Erythrobasidium* and *Cladosporium* showed significant abundance differences, being overrepresented in infected and symptomless samples, respectively. On the other hand, *Acinetobacter* (overrepresented in symptomless samples) and *Sphingomonas* (overrepresented in infected samples) were the two most highly abundant significantly changed bacterial genera with the highest fold change. *Gluconobacter*, *Pseudomonas* and *Geobacillus* were also observed among DA genera. The importance of both approaches - conventional microbiological techniques for cultivating microorganisms *in vitro* and metabarcoding - was demonstrated, as high-throughput sequencing alone did not detect all microbial species. Moreover, the morpho-anatomical analysis revealed the presence of calcium oxalate crystals in palisade cells indicating a response to biotic stress. In conclusion, it is noteworthy that members of several DA genera identified in this study have already been recognized as beneficial microorganisms. *Acinetobacter* plays a significant role as a plant-growth promoting bacteria. Similarly, members of the genus *Sphingomonas* have demonstrated antagonistic activity against several plant pathogens.

KEYWORDS: *Venturia oleagina*; metabarcoding; mycobiota; bacteriobiota

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IL43

RESEARCH INTO THE APPLICATION OF BACTERIAL-BASED BIOFORMULATIONS IN THE CONSERVATION OF FUNGAL-DETERIORATED WORKS OF ART IN SERBIA

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Fungi are considered one of the main causes of deterioration of all types of old masterpieces and a source of occupational hazard for conservators and restorers. Worldwide discovery and implementation of appropriate minimally invasive and environmentally friendly methods for control of fungal infestation is still a substantial problem. This is accentuated since the application of efficient chemical biocides is increasingly discouraged due to their toxicity, non-selective mode of action, abrasiveness, low long-term effectiveness, and promotion of resistance. Nowadays, a new paradigm in research sheds light on beneficial bacteria as the source of newly designed formulations with the potential to be implemented as an alternative to overused chemicals. Based on the up-to-date research, beneficial bacteria of *Bacillus* and *Pseudomonas* genera were shown to possess the highest potential to be used as a promising green alternative for sustainable and long-term cultural heritage conservation. Selected strains of these genera are saprobic, produce a high yield of a set of complex bioactive secondary metabolites, and are easy for cultivation and manipulation, making them ideal candidates for large-scale production of biocompounds in industrial reactors. Contemporary, a pioneer *in vitro* study in Serbia have shown the great potential of bacterial-based formulations to suppress the growth of deteriogenic fungi isolated from numerous infested and endangered works of art. Furthermore, the next phase of research, on infested laboratory mock models in simulated conditions, has confirmed strong fungicidal potential and most importantly demonstrated a lack of any unfavourable impact of applied bioformulations on treated material. In perspective, the final step entails the *in situ* application of the most promising bioformulation, by which the most adequate application method and long-term effectiveness assessment would be determined.

KEYWORDS: artifacts; beneficial bacteria; biodeterioration; fungal infestation; safeguard

IL44

ENVIRONMENTAL SPREAD OF ANTIBIOTIC RESISTANCE – THE ROLE OF INDUSTRIAL, AGRICULTURAL AND MUNICIPAL WASTE

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Antibiotic resistance (AR) is one of the greatest global threats to public health. The environment plays an important role in AR, as it serves as a reservoir and transmission route for resistant bacteria and resistance genes, as well as a source for the emergence of new forms of resistance. Understanding and addressing the environmental dimension of AR is therefore crucial for developing holistic strategies to mitigate the impact and protect public health. In my lecture, I will present data from our own studies, which we conducted in collaboration with several other laboratories. These included various environmental matrices impacted by diverse activities, such as industrial activities involving antibiotics (specifically, pharmaceutical wastewater and its effects on receiving river) as well as agricultural practices excluding antibiotics (studying soil treated with manure from antibiotic-free cows). We also investigated the dynamics of AR in municipal wastewater treatment plants. We used liquid chromatography/tandem mass spectrometry to investigate the extent of antibiotic pollution, as well as functional metagenomics, quantitative PCR, and exogenous plasmid isolation to investigate the resistome of environmental microbial communities. We show that high levels of macrolide antibiotics in river sediments are associated with increased abundance and mobility of macrolide resistance genes. Furthermore, our results show that the increase in ß-lactam-resistant bacteria observed in manure-treated soil originated from pre-existing reservoirs in the soil itself and not directly from the manure. Additionally, our results show that treated municipal wastewater is an important source of hazardous carbapenem-resistant enterobacteria that carry several carbapenemase genes simultaneously. Notably, we have identified the colistin resistance gene *mcr*-4.3 in pundrug-resistant *K. pneumoniae* from wastewater for the first time. It´s found on the conjugative IncHI1B plasmid, raising serious concerns about the dissemination of resistance to last-resort antibiotics. All these findings underline the urgent need for risk reduction measures in the identified environmental hotspots.

KEYWORDS: antibiotic resistance; soil; manure; pharmaceutical industry; wastewater

IL45

HARNESSING FUNGAL-BACTERIAL INTERACTIONS TO IMPROVE PLANT GROWTH AND HEALTH

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Microbiome analyses showed that fungi and bacteria cohabit in the plant environment. Separately they have been intensively studied for their impact on plant growth and health. Except some cases, little attention has however been paid to the intricacy of symbiotic fungi-bacteria associations.

Using different models, we described some interactions between fungi and bacteria, including a mycorrhiza-like fungus, a saprophyte and a phytopathogenic fungus. The mycorrhiza-like fungus *Serendipita indica* holds capabilities to enhance plant growth and to confer resistance to different stresses, but it is also known to host a symbiotic bacterium, *Rhizobium radiobacter*. However, its association with other bacteria and the potential positive effects on the fungus had not been explored. We analyzed how co-inoculations of bacteria and *S. indica* influence plant growth and protection against fungal pathogens. Bacteria and the fungus seem to cooperate in the process of root colonization and some bacteria enter inside the fungus. Another recent discovery was made on an ascomycetous saprophyte. We determined that specific fungal species host bacteria through isolation, metabarcoding, curing approaches and advanced microscopy techniques. Interestingly, all fungal isolates from a same species contain several similar bacterial taxa but differences are found between fungal species. Similar approaches were applied for a basidiomycetous wood pathogen, using a large set of fungal isolates from diverse worldwide origins. The existence of a core endohyphal bacteriome was detected with functions that remain to be explored. Besides highlighting the importance of multipartite microbial interactions, we discuss implications of our results for the development/application of microbial consortium-based bioprotectants and biostimulants as well as for the understanding of fungal pathogen-bacteria associations.

KEYWORDS: endofungal bacteria; microbe-microbe interactions

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IL46

IMPACT OF UNTREATED WASTEWATERS ON THE MICROBIOLOGICAL WATER QUALITY OF THE DANUBE RIVER AND ITS TRIBUTARIES IN SERBIA

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Pollution of surface waters still represents one of the major environmental challenges in the Republic of Serbia, where wastewaters are discharged directly into the recipients without proper treatment. Within the last decade, we have assessed the microbiological water quality of more than 100 sites situated at different water bodies in Serbia. Quality was determined using faecal indicator bacteria, while microbial source tracking was employed to assess the source of pollution. Almost 50 % of the investigated sites were characterized by critical or even higher level of faecal contamination indicating that untreated wastewaters indeed represent significant pollution pressure on surface waters. Human-associated markers were prevalent in samples from the majority of contaminated sites but the source of pollution was not exclusively human-associated. The impact on the water quality of the Danube River was demonstrated in our previous research conducted at the whole river level within the Joint Danube Surveys. In the river stretch from Novi Sad to its confluence with the Velika Morava River, all the midstream samples were critically polluted. In this section, the highest level of pollution was recorded downstream of Belgrade. As ultimate recipients of wastewaters, Danube and its largest tributary Sava currently represent the only solution for disposing of wastewaters originating from the Serbian capital's 1,700,000 inhabitants. Such kind of disposal rises additional issues such as antimicrobial resistance and presence of infectious agents in water. For instance during COVID-19 pandemic, we have demonstrated that SARS-CoV-2 RNA can be detected even in surface waters of the Danube River at the sites receiving high wastewater loads from Belgrade which was the unique case for the Basin. Despites its extreme importance in this case, wastewater-based epidemiology is neglected in our country and hereby we would like to emphasize the need for implementation of program of such kind in Serbia.

KEYWORDS: Danube river; wastewater-based epidemiology; faecal indicator bacteria

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IL47

ECOLOGICAL SERVICES OF BENEFICIAL MICROORGANISMS AS A PARADIGM OF SUSTAINABLE AGROECOSYSTEMS

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The global population demand for food forces intensive farming practices that guarantee high yields. The downsides of these practices have triggered the urge for a sustainable agriculture concept that highly values soil microbiota. Ecological services provided by beneficial microorganisms affect plant growth and yield, plant nutrition, mitigation of stress responses and resistance, etc., and can be exploited at different phases of plant growth, from germination to mature stage. Intervention at early stages through seed biopriming is an effective way to support seedling development and to utilise the full potential of the microbial ecological services. Biopriming of sugar beet, soybean, and white mustard seeds with *Azotobacter chroococcum* and *Bacillus* spp. provided vigorous seeds with increased germination, emergence, dry biomass, chlorophyll, and flavonoids content, capitalizing on the establishment of beneficial interactions. Intervention in the early stages of forestry seedlings growth strongly support an agroforestry approach that provide high-quality, resistant seedlings. Inoculation of one-year-old *Platanus* x *acerifolia* and *Robinia pseudoacacia* L. resulted in higher seedlings with wider root collar diameter, and higher total dry biomass, higher levels of total soluble proteins, and chlorophyll. Biofortification effectively exploits microbial ecological services and raise micronutrient availability to plants. Cultivation of *Trichoderma* spp. in Na2SeO3-enriched nutrient medium resulted in fungal biomass with a significantly higher Se content (642.60 µg/g) compared to control (4.43 µg/g). The biomass applied as an organic biofertilizer on wheat plants resulted in a higher Se in roots and shoots of the treated plants, compared to the control indicating that the application of useful microorganisms contributes to the quality of the product. Ecological services provided by soil microorganisms affect plant growth, development, resilience, yield, and quality. They are incorporated in different components of sustainable agriculture such as manuring, bioreagents addition, biofortification, and agroforestry etc. and represent valuable resource built into the basis of sustainability.

KEYWORDS: agroforestry; beneficial microorganisms; biofortification; inoculation; sustainable agriculture

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IL48

INTER-SPECIES BACTERIAL SIGNALLING IN THE PLANT MICROBIOME

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Plant associated microbial communities have key roles in biotic and abiotic stress tolerance and nutrient acquisition. Plant-microbe associations occur at the leaf aerial parts in the phyllosphere and at root level in the rhizosphere. The rhizosphere (the nearest soil area to the roots) hosts a rich microbial plant community which provides a series of beneficial outcomes related to plant growth. Plant roots recruit their rhizosphere microbiome from bulk soil and a small number enter colonising the root endosphere and some then move to other plant organs. Proteobacteria are amongst the most abundant (approximately 50%) and diverse microbes found in plant rhizosphere microbiomes. Quorum sensing (QS) is a major form of cell-cell contact-independent signaling used by bacteria to regulate group behaviors. The most common QS system in proteobacteria thus far uses N-acyl-homoserine lactone (AHL) signals, which are synthesized by the LuxI-family AHL-synthase and sensed by a cognate AHL-binding LuxR-family receptor. Recent studies from my laboratory has evidenced the predominance of LuxR 'solos' in rhizosphere proteobacteria which lack the genetically adjacent cognate LuxI-type synthase. LuxR solos maintain the modular structure of canonical QS LuxRs, with some retaining AHL-binding capabilities while others have evolved to bind to other signals including plant-derived. The role of some LuxR solos and their potential of becoming a major regulatory family involved in cell-cell signaling in the plant microbiome is discussed.

KEYWORDS: bacteria; plant; microbiome; quorum sensing; LuxR

OF49

ENHANCING BIOREMEDIATION EFFICIENCY: NOVEL ISOLATION TECHNIQUES FOR MICROBIAL CONSORTIA IN RECALCITRANT PAH-CONTAMINATED SOILS

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Contamination of soils with hydrocarbons, particularly recalcitrant Polycyclic Aromatic Hydrocarbons (PAHs), poses a significant environmental challenge. The structure of PAHs amplifies the complexity of soil remediation. Seeking sustainable solutions, bioremediation approach, specifically by employing microbial consortia, becomes prominent. However, conventional isolation techniques for consortia, while time-consuming, present a suboptimal outcome. The classical methods risk losing co-dependent species, diminishing the overall efficiency of the microbial community. In light of this, we introduce a novel method for isolating bacterial consortia from PAH-contaminated soil. Our approach aims to enhance efficiency by preserving co-dependent relationships often lost in traditional isolation techniques. We subsequently compare the degradation efficiency of our isolated consortia with that of synthetic consortia obtained from the same soil. In our methodology, PAHs were extracted from contaminated soils. Consortia were then isolated using two innovative techniques: Interspecies Aggregation Combinatorics Microfluidic Entrapment (iACME) and u-Lure in Microfluidics and Entrapment (uLure). Characterization of the isolated consortia was performed using fluorescent microscopy and flow cytometry. Subsequently, these consortia were loaded onto porous carriers, immobilized with hydrophobic alginate, and tested for their ability to degrade native PAHs using GC-MS analysis. The obtained results were compared with the degradation efficiency of a synthetic consortia comprising 8 strains. Our experimental findings indicate that the application of different isolation methods resulted in the isolation of strains with significantly distinct compositions and PAH-degradation efficiencies compared to synthetic consortia.

KEYWORDS: bioremediation; isolation technique; iACME, PAH

ACKNOWLEDGEMENT: The authors acknowledge financial support from the Slovenian Research Agency (program No. P1-0143, project no. J7-9400, J1-3033, J4-4561, J4-4556), European Commission (grant no. 826312, 952379 and 101060211), project CROSSING (grant PIE - 0007) and European Regional Development Fund - UIA, grant no. UIA02-228.

OF50

SOIL MICROBIOME DIVERSITY IN MAIZE-WINTER WHEAT CROP ROTATION

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The soil microbiome controls key functions in agroecosystems determining soil fertility, crop productivity, and stress tolerance. Crop rotation is one of the oldest agricultural practices that has a positive effect on soil quality and the control of weeds, pests, and pathogens. New insights into soil microbiome confirm the positive influence of crop rotation on the diversity of the microbiome. This study investigates the effects of different crop rotations under conventional fertilization/weed management practices on soil microbial diversity and community structure in two of the most commonly grown crops in Serbia. The study investigated the bacterial population in samples from long-term cultivation experiment of maize continuous cropping (M-CC) and maize-winter wheat rotation (M-WW) at two-time points (December 2022 and May 2023). The results of 16S rDNA amplicon community profiling and beta diversity analysis showed clear clustering depending on season, cropping sequence, and herbicide application. The results show that the composition of the bacterial community in soil is largely influenced by seasonal changes. When comparing bacterial communities in the same season (May), we observed a significantly higher biodiversity in M-CC compared to M-WW soils, suggesting that type of agricultural practice has a significant impact on the composition of the microbiome by influencing selection and survival of specific microbial taxa. In addition, a significant shift was observed between the bacterial community composition in M-CC samples under herbicide treatment and in control soils in December, while the M-WW community was stable under all conditions tested. Taxonomically, *Actinobacteria* dominated the soil microbiome under all conditions (53%), followed by *Proteobacteria* (23%), *Acidobacteria* (15%), and *Firmicutes* (8%). Despite fluctuations in relative abundance, some interesting taxa, including *Bacillus*, *Microlunatus,* and *Blastococcus*, dominated the soil microbiome under all conditions. These data provide insights into microbial dynamics, integrating the cultivation methods with metagenomic approaches to evaluate different cropping practices.

KEYWORDS: crop rotation; microbiome; community profiling

GR51

ASSESSMENT OF GROWTH-PROMOTING PROPERTIES OF *PSEUDOMONAS* SPP. ON SOYBEANS UNDER FIELD CONDITIONS

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The soybean is recognized worldwide as one of the most important crops due to its protein- and oil-rich seeds. Several beneficial bacterial strains, including those of the genus *Pseudomonas*, are known to increase plant yield and disease tolerance. The aim of this study was to test the potential of 15 strains of *Pseudomonas* spp. to promote soybean growth under field conditions. Strains were identified based on 16S rRNA and gyrB sequences, and strains belonging to risk group 1 were further analyzed. Selected strains were tested for plant growth-promoting (PGP) properties, biofilm formation and *in vitro* antimicrobial activity against various fungal pathogens. PGP activity indicators of the selected strains were evaluated on soybean plants grown in fertilizer-free soil and in soil treated with 70% and 100% fertilizers. Three *Pseudomonas* spp. strains, *P. putida* VB131A, *P. fulva* VB134B and *P. rhodesiae* VB143S, were selected as PGP candidates. All three strains showed the ability to produce IAA and solubilize phosphorus, while only *P. rhodesiae* VB143S showed strong lipase and protease activity. Only *P. fulva* VB134B showed the ability to produce biofilm and hydrogen cyanide. All three strains showed *in vitro* antifungal activity against *Alternaria infectoria*. *P. fulva* VB134B and *P. rhodesiae* VB143S showed antifungal activity against *Alternaria alternata* and *P. putida* VB131A and *P. rhodesiae* VB143S against *Epicoccum nigrum*. Only *P. fulva* VB134B showed activity against *Monilinia laxa*. Statistically significant differences in plant height were not observed regardless of the strain or soil treatment used. In terms of first pod height, the *P. fulva* VB134B treatment was most effective when applied to soybeans grown in 100% fertilized soil. In contrast, plants treated with *P. rhodesiae* VB143S and grown in 100% fertilized soil had the highest number of branches and pods per plant.

KEYWORDS: *Pseudomonas* spp.; soybean; plant growth-promoting properties.

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GR52

INCREASING EFFICIENCY OF BIOPHOTOELECTROCHEMICAL PROCESSES BY STRUCTURING BIOFILMS OF AEROBIC PHOTOTROPHIC MICROORGANISMS

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This research aims to develop innovative methods for preparing structured biofilms in biophotoelectrochemical systems, utilizing various aerobic phototrophic microorganisms. The study focuses on enhancing the efficiency of biofilm formation on electroactive surfaces, such as those found in microbial fuel cells. Different attachment methods for these microorganisms will be systematically tested and characterized to understand their behavior on various surfaces. The primary goal is to establish a robust and efficient technique for immobilizing the microorganisms, allowing for their sustained activity on electroactive substrates. This process involves the exploration of electrode modification techniques and the optimization of conditions for the attachment of the specified microorganisms. The study will employ advanced characterization techniques, including fluorescent microscopy and flow cytometry, to analyze the structured biofilms. Additionally, the immobilized biofilms will be subjected to thorough testing for their ability to generate electrical current and other relevant electrochemical parameters. The research methodology will involve comparisons between different attachment methods and an evaluation of the performance of the structured biofilms in biophotoelectrochemical systems. The significance of this work lies in its potential to advance the understanding of how aerobic phototrophic microorganisms can be effectively utilized in biophotoelectrochemical systems, paving the way for applications in sustainable energy production and environmental monitoring. The outcomes of this research may contribute valuable insights into the development of biofilm-based technologies for diverse electroactive surfaces, fostering innovation in the field of biophotoelectrochemical systems.

KEYWORDS: attachement; structured biofilms; biophotoelectrochemical systems

GR53

CRYOPRESERVATION-BASED STRATEGY FOR PPV ERADICATION IN AUTOCHTHONOUS PLUMS: INSIGHTS FROM FRUIT RESEARCH INSTITUTE ČAČAK

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The importance of conserving plant species, particularly those crucial for agriculture, has surged to ensure sustainable utilization of biological resources and prevent further loss of plant diversity. Cryopreservation, the most advanced method for plant conservation, offers promise in this regard. It can be employed for conservation per se, or as a potential tool (cryotherapy) for pathogens elimination from infected plants, particularly viruses that threaten agricultural productivity. In the recent study, two vitrification-based cryopreservation methods, D and V cryo-plate, were evaluated for their efficacy in eradicating plum pox virus (PPV) from autochthonous plum cultivars 'Crvena Ranka' and 'Belošljiva', widely present in the Balkan region. A total of 111 pool samples of in vitro shoots of plums 'Belošljiva' and 'Crvena Ranka' were tested (65 and 46 samples, respectively). Each sample consisted of an average of 10 plants, in total about 1100 plants. Health status of *in vitro* shoots originated from both control (non-frozen explants) and cryopreserved explants of infected plums were continuously tested during multiplication for the PPV presence with conventional reverse transcription polymerase chain reaction (RT-PCR). To evaluate these results and obtain the highest efficiency of detection, all samples were further tested using Real-time PCR (qPCR). Using RT-PCR, the PPV was detected in 67 out of 111 tested samples: in 39 samples of plum 'Belošljiva' and 28 of plum 'Crvena Ranka'. Using a qPCR assay, PPV was confirmed in 82 tested samples. Effective PPV elimination from 'Crvena Ranka' was achieved through three cryo-treatments, while neither method (D and V-cryoplate) nor the applied treatments led to PPV elimination in 'Belošljiva'. The qPCR assay demonstrated higher sensitivity compared to conventional RT-PCR, highlighting its potential utility in such assessments. Our findings supported the exceptional specificity and sensitivity of the qPCR technique in detecting PPV in *in vitro* shoots of the plums 'Belošljiva' and 'Crvena Ranka' post-cryotherapy.

KEYWORDS: autochthonous plums; plum pox virus; cryotherapy; qPCR; efficiency

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GR54

IDENTIFICATION OF CULTURABLE BACTERIA ASSOCIATED WITH THE RHIZOSPHERE OF *LABLAB PURPUREUS* GROWING IN NAMIBIA

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Dolichos lablab (*Lablab purpureus* (L.) Sweet) is a multipurpose drought tolerant protein-rich legume crop native to Africa and grown in warm temperate to tropical climates for its edible seeds and manure. *Lablab purpureus* holds significant benefits to subsistence farmers and offers a great promise for sustainable crop productivity, especially in marginalised areas. Its uses range from human consumption as a vegetable to improving soil fertility, and as forage. Notwithstanding *Lablab purpureus* crucial potential functions in Namibia, there is currently limited information regarding the plant's rhizosphere bacteria. The study aimed at identifying *Lablab purpureus'* natural rhizosphere bacteria. Isolation of rhizosphere bacteria involved the use of general media (Luria Bertani agar and tryptic soy agar); selective media such as Rhizobium and Yeast Extract Mannitol (YEM) Congo red from soil sample extracts. Eighty-five strains of bacteria were isolated and were subsequently identified by 16S rRNA gene sequencing analysis. The results showed that they belonged to the following genera, *Bacillus, Streptomyces, Exiguobacterium, Stutzerimonas, Rhizobium, Acidovorax, Agrobacterium, Psychrobacter, Priestia, Planococcus, Bhargavaea, Stenotrophomonas, Caulobacter, Peribacillus, Niallia, Athrobacter, Sphingobium, Enterobacter, Sphingobacterium, Sinorhizobium, Flavobacterium, Microbacterium, Metabacillus, Neobacillus,* and *Pseudomonas* which are reported to have growth promoting substances. The study highlighted the potential use of these plant growth promoting rhizobacteria for inocula production or biofertilisers for enhancing growth and nutrient content of beans and other crops under field conditions. The study was the first report of *Lablab purpureus's* rhizosphere associated bacteria in Namibia.

KEYWORDS: rhizosphere bacteria; *Lablab purpureus*; Dolichos lablab; 16s rRNA; Namibia

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GR55

UNVEILING MICROBIAL ENIGMAS: EXPLORING THE POTENTIAL OF FLOW CYTOMETRY IN MICROBIOLOGY

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Flow cytometry has emerged as a transformative technique in microbiology, enabling researchers to analyse and sort cells swiftly, thereby revolutionizing our understanding of microbial populations. Microbes, ubiquitous in nature, profoundly impact diverse fields including human health, ecology, and industry. Traditional microbiology techniques, reliant on culturing methods, offered limited insights into microbial diversity due to their inability to culture a vast majority of species and their snapshot-based approach. In contrast, flow cytometry facilitates real-time analysis of microbial communities, overcoming the constraints of culturing techniques. By employing principles of light scattering and fluorescence, flow cytometry enables the characterization of individual microbial cells, including unculturable species. Moreover, it provides a dynamic understanding of microbial ecosystems and their responses to environmental stimuli. The application of flow cytometry extends across various domains such as microbial ecology, environmental monitoring, and medical diagnostics. In microbial ecology, it unravels the complexities of microbial communities, aiding in understanding nutrient cycling and ecosystem functions. Additionally, in medical microbiology, flow cytometry's ability to swiftly identify and characterize pathogens revolutionizes infectious disease diagnosis and treatment. Looking ahead, the future of flow cytometry in unravelling microbial mysteries appears promising, especially in exploring the human microbiome and leveraging emerging technologies like single-cell sequencing. The ongoing automation and miniaturization of flow cytometry technology promise wider accessibility and adoption across industries and research settings, fostering advancements in microbiology and contributing to human health and environmental sustainability. In conclusion, embracing flow cytometry as a powerful tool in microbiology promises to drive scientific discoveries, unveil microbial secrets, and propel advancements in human health and environmental conservation.

KEYWORDS: flow cytometry; microbial populations; microbial ecology; environmental monitoring; medical diagnostics

GR56

PROMOTION OF PEPPER GROWTH USING *ENTEROBACTER CLOACAE* AND *PSEUDOMONAS PUTIDA* UNDER GREENHOUSE CONDITIONS

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Beneficial plant growth promoting rhizobacteria (PGPR) are able to promote plant growth, produce secondary metabolites to control phytopathogenic diseases and induce plant resistance. In order to reduce the use of chemical fertilizers and pesticides, many researchers offer alternatives for sustainable agriculture- the application of biofertilizers. The aim of this study was to examine the influence of the rhizobacteria *Enterobacter cloacae* and *Pseudomonas putida* on pepper growth under greenhouse conditions. The bacterial isolates were tested (in vitro) for their physiological, biochemical (catalase test, urease test, nitrate reduction, citrate utilization and production of hydrolytic enzymes) and plant growth promotion- PGP characteristics (production of ammonia, siderophores, hydrogen cyanide, biofilm, indole acetic acid and phosphate solubilization). The influence of the bacterial inoculum on initial plant growth was investigated under greenhouse conditions on plastic trays. The morphological parameters of the plants were measured after 35 days: plant height (mm), root length (mm) and weight of fresh and dry plant biomass (g). Data were analyzed using SPSS software and One-way analysis ANOVA with Tukey´s test was performed. The results showed a significant difference in plant height and biomass between the treatments and the control (non-inoculated plants), suggesting the possibility of using characterized isolates as biofertilizers.

KEYWORDS: plant growth promotion; rhizobacteria; pepper

GR57

COMPARISON OF PHYSICO-CHEMICAL PARAMETERS AND ASSOCIATION OF FUNGI, BACTERIA AND ACTINOMYCETES OF WET AND DRY COMPOST

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Composting is the process of decomposing bio-waste with the help of living organisms to produce carbon dioxide, water, heat and compost. It is the oldest and most natural way of recycling waste. A relatively simple way, through recycling that is environmentally friendly, after which we return the waste back to use as an ecological fertilizer. The main objective of this research was to study the physico-chemical properties and the microbial diversity of wet and dry compost. This research covers two parts of compost experiments. The first part deals with the determination of pH, moisture, total number of bacteria, yeasts and molds, as well as determination of the number of Bacillus spp. and actinomycetes in compost. The second part refers to the isolation of pure crops of strains of Bacillus spp., which significantly contribute to the process of biodegradation of organic waste and its transformation into compost. The moisture content values ranged from 26-91% and the pH value ranged from 7.77 – 7.90. The total organic matter values ranged from 22.15 to 24.43 %. The total nitrogen values ranged from 2.76 to 0.65 %. The total potassium values ranged from 22.74 to 30.48 % respectively, for wet and dry compost. The wet compost had a greater number of fungi, bacteria and actinomycetes.

KEYWORDS: physical properties; chemical properties; microbial communities; organic waste

GR58

HARNESSING *BACILLUS* SPP. FOR TARGETED BIOCONTROL OF *SCLEROTINIA SCLEROTIORUM*

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Sclerotinia sclerotiorum, a devastating fungal pathogen, poses significant challenges in managing diseases across various plant species, including economically significant crops worldwide. This plant-pathogenic fungus, distributed across temperate, tropical, and arid regions, causes severe crop damage, leading to substantial yield losses. Given the limitations of synthetic agents for its suppressing, attention has turned towards biological control methods, particularly the selection of beneficial microorganisms. Therefore, the aim of this research was to detect antagonistic effect of bacterial isolates against *S. sclerotiorum*. Seventeen bacterial isolates isolated from soil (PAM2.1 – PAM 2.17) were screened for their antagonistic activity against this plant pathogen *in vitro*, on PDA medium. Additionally, bacterial traits significant for the biocontrol, such as the production of siderophores, amylase and cellulases were assessed on appropriate solid media, while the presence of genes coding for antibiotics (fengycin, surfactin, iturin C, subtilin, and bacilysin) was assessed by PCR method. *Further,* for the most effective isolate the *amplification of* DNA sequence coding for 16S rRNA gene was done using P0/ P6 primer pair. For the identification, the obtained sequences were compared to those available in the National Center for Biotechnology Information (NCBI) database. Out of 17 tested bacterial isolates, only one (isolate PAM 2.2) showed antagonistic effect against *S. sclerotiorum*, with inhibition of mycelial growth of about 71%. The same isolate showed the ability to produce siderophores, amylase and cellulase (CMCase). Further, the presence of genes coding for surfactin, subtilin and *bacillomycin was detected for the isolate PAM2.2.* Based on the NCBI BLASTn analysis of the 16S rRNA gene sequence, isolate PAM2.2 was identified as *Bacillus* spp. This research highlights isolate PAM2.2 as a promising candidate for biological control against *S. sclerotiorum*, demonstrating significant antagonistic effects, production of relevant enzymes and antibiotics, thus offering a potential sustainable solution for disease management in agriculture.

KEYWORDS: *Sclerotinia sclerotiorum*; *Bacillus* spp.; antibiotic production; fungal inhibition; siderophore producers

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GR59

ETHNOBOTANICAL STUDY OF SELECTED *ARTEMISIA* SPECIES AS A GUIDE FOR NEW ANTIMICROBIALS

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The main objective of the present study was to collect information on traditional uses of *Artemisia* species for medicinal purposes, especially against human parasites and pathogenic bacteria and fungi. The ethnobotanical research was conducted in all (east, west, central, south, and north) regions of Serbia, from June to November in 2023. In total, the investigated area includes 64 localities (24 cities and 40 villages). Data were collected through semi-structured interview from the 376 inhabitants. According to our results only *A. absinthium* L. (wormwood), and *A. annua* L. (sweet wormwood) were recognized as a source of antimicrobial and antiparasitic agents. Local population in the cities use dried flowering aerial parts of both species in the form of infusion or tincture against parasites, viruses, bacteria and fungi. Wormwood was used for these purposes only in urban areas in southern Serbia, where informants particularly mentioned usage of this plant in the treatment against *Candida* spp. On the other hand, sweet wormwood was used only in urban areas in eastern Serbia. Obtained results are congruent with some previous ethnobotanical studies conducted in Bulgaria, Romania and Croatia where, besides their other bioactivities, both *A. absinthium* and *A. annua* were mentioned as anthelmintic and antimicrobial agents. Therefore, we can concluded, that similar ethnobotanical studies can lead to discovery of new antimicrobials in the future.

KEYWORDS: wormwood; sweet wormwood; microorganisms; parasites, pathogens

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GR60

HYDROPHOBIC BACTERIAL CARRIER APPROACH TO BIOREMEDIATION OF PETROLEUM OIL POLLUTED SOIL

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Oil contamination is a persistent environmental issue mainly due to the toxicity of oil and the recalcitrant nature of hydrocarbons. Bioremediation approaches involve biostimulation, bioaugmentation and other methods, targeting the additions of nutrients and microbes to perform the degradation and the additions of surfactants to make oil more bioavailable. While admirable results can be achieved, long chain hydrocarbons (< C30) and polycyclic aromatic hydrocarbons (PAHs) are highly hydrophobic and largely unaffected by the conventional bioremediation approaches. To achieve high substrate availability and to enable potential co-metabolic pathways between bacteria, we propose a novel hydrophobic matrix with embedded bacteria for the degradation of petroleum polluted soil. To achieve a high surface area of the matrix to be in the contact with the polluted soil, solid support in form or organic and inorganic material was used and covered with bacterial biomass in alginate matrix. Surface of the matrix was altered to have hydrophobic properties, making the substrate more bioavailable to degradation. The prepared carriers were incubated in the contaminated soil and concentration of hydrocarbons was measured, along with testing of the carriers regarding the absorption capacity and metabolic activity using oil degradation redox indicator. The results showed organic solid support material exhibits greater absorbency compared to inorganic solid support material. This was evident by higher reduction in the signal of the redox indicator and this relationship was further confirmed by a high correlation coefficient ($r = 0.91$). Using hydrophilic alginate as coating on the carriers showed no reduction of the redox indicator, contrary to the hydrophobic carriers. GC-FID analysis confirmed sterile hydrophobic carriers reduce the amount of hydrocarbons in soil, while carriers with embedded bacteria reach up to 92 ± 30 % of degradation in 40 days. The novel approach of hydrophobic matrix with embedded bacteria showed promising results of hydrocarbon degradation and the advancements that can be achieved in comparison to alternative methods of bioaugmentation, where bacteria are added in liquid form.

KEYWORDS: bioremediation; oil pollution; hydrophobic matrix; oil degrading bacteria

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GR61

BIODEGRADATION OF HEXACHLOROCYCLOHEXANE (HCH) BY BACTERIAL AND FUNGAL CONSORTIA IN SEMI-CONTROLLED SOIL AND SLURRY MODELS

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Hexachlorocyclohexane (HCH) represents a group of organochloric isomers; historically used for agricultural pest control across the globe. Like most organochloric pesticides of the past, their toxicity, carcinogenicity and tendency to bioaccumulate and cross-infiltrate neighbouring food chains has led to their discontinued production. However, the issue of mediating and resolving already infected and contaminated agricultural and non-commercial ecosystems remains ongoing. Several countries like Spain, India, North Macedonia and the Netherlands have employed brute-force physical and chemical methods of eliminating large landfills of HCH by extraction and incineration, alkaline treatment or a combination of both. Despite these efforts, soil and water residues can still be detected even at great distances from the locations that these landfills once occupied. The contemporary consensus is that these minute, yet pernicious residues must be dealt with by subtler and more cost-efficient means. Bioremediation using microbial communities in particular has received momentous praise these past two decades. In an effort to produce such a solution to aid the remediation of N.Macedonia's contaminated agricultural surfaces, bacterial and fungal strains were isolated from HCH contaminated soils and adapted to incrementally increasing HCH concentration in-vitro. These isolated strains were then combined to form unique microbial consortia which were then evaluated in semi-controlled laboratory soil and slurry models. Microbial growth was observed in both the fungal and bacterial consortia in both media. The concentration of HCH, as measured using HPLC technology, was shown to be reduced up to %42 of the original concentration within 10 days of incubation. The reduction in observable HCH residues was observed to be greater in the slurry model compared to the dry soil model, indicating potentially interesting ways to stimulate these processes in-situ with similar or identical consortia. Overall the results of this study showed great promise for the future of HCH bioremediation in N.Macedonia.

KEYWORDS: lindane; microbial biotechnology; soil bioremediation

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GR62

SOIL ARSENIC AND NUTRIENTS DYNAMICS AND AVAILABILITY AFFECT MICROBIAL DIVERSITY, AND PLANT-MICROBE INTERACTIONS: CONNECTING AGRONOMY TO GENOMICS

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Alluvial soil is enriched in nutrients that helps to maintain a healthy plant-microbial symbiosis. This six-yearlong field-based study focuses on the As dynamics in agricultural soil affecting the rice productivity, plant physiology, and altered soil microbial communities under differential cultivation approaches. Soil macro-micronutrients were also found to modulate the microbial communities while expressing differential As-resistance and metabolizing genes in the soil systems. Metagenomics and gene-specific High Throughput sequencing (HTS) of predominant soil microbes with Gene Ontology (GO) profiling, KEGG metabolism pathways assessment, Panther-pathways analysis, microbial network distributions were performed in every season of each year to understand how As and soil nutrient dynamics conjugatively influence the dominant soil microbiome. Such altered microbial profile also induce plant As responsive genes and biochemical enzyme productions under changed soil conditions. This first-of-its-kind study indicated that the drying-wetting (DW) irrigation, compared to the continuously flooding (CF), can retain a redox status of soil optimal for the soil microbes to thrive while releasing the greatest pulses of bioavailable nutrient pool. Such burst of nutrients triggers the plant's growth and reduces the generation of stress markers even under high As contaminated soil Metagenomics and HTS of microbes showed that *ars* operon and *aio-arr* gene clusters were mostly up-regulated in the CF fields to tackle the high As availability. DW field analyzed microbes expressed these genes in a lower degree with greater expressions of central carbohydrate metabolism, multivitamin-cofactor synthesis, nitrogen-sulfur metabolism and amino acid synthesis, as analyzed via GO and KEGG modules. Microbial network analysis from DW samples indicated greater species enrichment, α/β indices with higher relative abundance and rarefaction analysis. Rice harvesting index from FL and DW fields suggested a prominent role of microbes in reduction of As stress and enhanced nutrient uptake. This whole study provides an important understanding of soil elemental influence on microbes to confer changes in crops.

KEYWORDS: soil microbes; arsenic; metagenomics; paddy

GR63

EFFECT OF NATURAL FARM INPUTS ON FRUITING, NUTRIENT CONTENT AND RHIZOSPHERE STOCHIOMETRY IN LEGUME INTERCROPPED STRAWBERRY (*FRAGARIA × ANANASSA* DUCH.)

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Natural Farming, the regenerative agriculture has proven the most suitable paradigm which offers a solution to boost farmers' income, ensure better soil health, reduce production costs and reduce water consumption. This agro-ecological farming based on on-farm biomass recycling and exclusion of all synthetic chemical inputs. Nutri-sensitive agricultural innovation can be a cost-effective approach to promote good health and well-being. The current study emphasized the potential impact of natural farm inputs on sustainable and high-quality crop production. Biological modifications namely, Ghan-jeevamrit and Jeevamrit have been used. Ghan-jeevamrit contained 4-5 days air dried indigenous cow dung (100 kg), raw sugar (1 kg), phosphorus solubilizing bacteria rich pulse flour (1 kg), cow urine (3 L) and undisturbed bunds/ forest soil (250 g). Liquid microbial culture of Jeevamrit contained cow dung-urine (pH-5.65, EC-0.23 dS/m) and the presence of *Azotobacter chroococcum*, *Pseudomonas* sp. and actinobacteria. The trial included, Ghan-jeevamrit-2.5 kg/m^2; Ghan-jeevamrit-5 kg/m^2; Ghan-jeevamrit-2.5 kg/m^2 + Jeevamrit-2.0 L/m^2; Jeevamrit-2.0 L/m^2; Ghan-jeevamrit-2.5 kg/m^2 + Jeevamrit-1.0 L/m^2 and FYM-100% of nitrogen equivalent basis. Ghan-jeevamrit-2.5 kg/m^2+ Jeevamrit-2.0 L/m^2 significantly improved productivity of strawberries. Microbial biomass of *A. chroococcum, Pseudomonas sp.* and arbuscular mycorrhizal fungi (AMF) were positive. Microbial formulations had build–up an increase of *A. chroococcum, Pseudomonas sp.* and AMF count received Jeevamrit and Ghan-jeevamrit. Increased dehydrogenase and acid-phosphatase enzymes were registered in rhizosphere. Principal component analysis revealed highest cumulative variation for *A. chroococcum, Pseudomonas sp.* and AMF population. The study concluded that application of Ghan-Jeevamrit-2.5 kg/m^2 + Jeevamrit-2.0 L/m^2 had a significant impact on production, quality metrics, *A. chroococcum, Pseudomonas sp.* and AMF count in rhizosphere. Bio-mobilization and recycling of native nutrients encouraged dehydrogenases and acid phosphatase enzymatic activity to maintain soil health and productivity for long-term and sustainable strawberry production.

KEYWORDS: natural farming; plant nutrition; rhizosphere microbiome

GR64

COMPARISON OF METHODS TO DETECT BIOSURFACTANT PRODUCTION BY BACILLUS SPP. ISOLATED FROM CONTAMINATED AND UNDISTURBED SOIL

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Biosurfactants are secondary microbial metabolites, which possess powerful surface-active capabilities, alongside a wide plethora of biological activities, and are considered a sustainable alternative for synthetic surfactants. That is why they are the subject of research for the oil, pharmaceutical, cosmetic and food industries. Very little is known about the distribution of biosurfactant producing microorganisms in contaminated vs. undisturbed soil. The goal of this research was to test the activity of biosurfactant producing Bacillus spp. Bacillus represents a ubiquitous genus, known for its presence in even the most extreme of environments, as well as for the many antimicrobial compounds it produces. Among them are the cyclic lipopeptides (CLP) - one of the most researched groups of biosurfactants. While analyzing the activity of the biosurfactants, efforts were also made to elucidate the different factors of the soils which could potentially affect it, for the duration of a whole year. Ohis served as a contaminated soil sample, while Karadzica served as an undisturbed one. Results showed no significant difference in frequency of biosurfactant producing Bacillus spp., with very small differences in the activity of their biosurfactants, despite a significant difference in soil acidity. There was no correlation between the methods used to assess the activity of the biosurfactants, as they measure different properties a biosurfactant can have. Those were the microplate method, drop-collapse method and emulsification index. Few of the isolates showed promising activity for further investigation in the direction of industrial use.

KEYWORDS: sustainable; cyclic lipopeptides; microplate method; drop-collapse method; emulsification index

PP65

CHARACTERIZATION, VIRULENCE AND ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM MEAT AND MILK IN ITALY

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Staphylococcus aureus is one of the major pathogenic microorganisms involved in the global threat of Antimicrobial Resistance (AMR) and the most prevalent foodborne pathogen found in animal-source foods. Milk, beef and pork meat are perishable raw food matrices that provide suitable media for *S. aureus* propagation. We compare 48 *S. aureus* strains isolated from raw milk and meat in Italy to assess their antibiotic resistance profiles, enterotoxin production, hemolytic activity, surface characteristics and virulence factors involved in infection. In addition, we correlate resistance/susceptibility to ampicillin, cefoxitin, and tetracycline of eight representative *S. aureus* strains with their surface characteristics and toxicity towards Caco2 cells. The bacterial surface is the first structure that interacts with the surrounding environment and host cells, and variations therein can influence antibiotic susceptibility. Additionally, AMR may be closely linked to the production of enterotoxins and other virulence factors by *S. aureus*, worsening its pathogenicity. Representative *S. aureus* strains showing mono or multi-resistance to ampicillin, cefoxitin and tetracycline were selected based on their AMR profiles determined phenotypically using the Disk Diffusion method. Additionally, the presence of resistant genes was confirmed by PCR. None of the selected strains had enterotoxin genes as verified by PCR. Electrophoretic mobility which is related to bacterial surface charge was assessed by the Zeta potential measurements, while Microbial Adhesion to Solvents was estimated through bacterial hydrophobic affinity. Ethidium bromide Influx Assay was performed to assess cell membrane permeability. Observed phenotypical and genetic characterizations were correlated to their adhesion to Caco2 cells. This study confirms that *S. aureus* foodborne isolates pose a risk to consumers. Although circulating strains were found to produce no enterotoxin, they can produce other virulence factors, and carry different AMR genes. The surveillance of this foodborne pathogen should be enhanced since it presents a high risk of infections and therapeutic failure.

KEYWORDS: antimicrobial resistance (AMR); food; *Staphylococcus aureus*; bacterial cell surface; virulence

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PP66

THE NUMBER OF PHYSIOLOGICAL GROUPS OF MICROORGANISMS AS AN INDICATOR OF THE ECOLOGICAL STATUS ALONG THE MOUNT BABA ALTITUDE GRADIENT

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Physiological groups of soil microorganisms play an important role in the regulation of ecosystem functions and cycle of organic matter. Mountains are excellent systems for studying microbial communities because of the difference in abiotic and biotic variables over a relatively short distance along an elevation gradient. This difference enables the analysis of the effects of high fluctuations of above-ground vegetation, local soil and climatic conditions in relation to the representation of soil microorganisms. Literature data show that individual groups of microorganisms are more sensitive than plants and animals to the effects of environmental changes, so changes in the number of microorganisms at different altitudes are considered a good indicator of the ecological status. Taking into account the limited knowledge about the impact of climate change on soil microorganisms in the mountains, the goal of the research is the identification of the factors that influence the number of different groups of microorganisms. According to the obtained results, it can be observed that low pH and temperature have a negative impact on the number of soil microorganisms, while the increased altitude and reduced % of humus reduces the number of microorganisms. The abundance has a negative correlation with the altitude, and a positive correlation with the % of humus.

KEYWORDS: soil; bacterial communities; elevation gradient; microbial ecology

PP67

AN INSIGHT INTO THE DISTRIBUTION AND ABUNDANCE OF BACTERIAL SPECIES ALONG DIFFERENT SIZE FRACTIONS OF THE SUSPENDED PARTICULATE MATTER AND THEIR CORRELATION WITH MERCURY LEVELS

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This research delves into the interplay between bacterial communities' development and the size of suspended particles present in freshwater. The aim is to unravel bacteria's natural distribution and abundance along size fractions ranging from 0.2 to 180.0 micrometres (µm), gaining insight into the relationship dependent on particle size. Particle size influences the surface area for bacterial colonisation as increased surface area can promote bacterial growth and activity. Particle size also influences the dispersion in aquatic environments – the size of particles to which bacteria attach can dictate the dispersal patterns in the water column. Furthermore, particle surfaces serve as attachment sites for bacterial biofilm formation and their size dictates the complexity and stability of biofilms. The subject of the study is a freshwater ecosystem, specifically a river, which is characterised by a strongly present pollutant, mercury. Thus, the research extends beyond bacterial characterisation, seeking to understand how a potent pollutant affects bacterial communities, focusing on size-dependent dynamics. Water samples will be subjected to size fractionation via filtration, and DNA will be isolated. Subsequent polymerase chain reaction (PCR) amplification and sequencing facilitate the targeted assessment of the genetic information in the microbial communities within size fractions. Furthermore, by gaining information on the concentration and distribution of mercury along size fractions, we expect to reveal size-specific adaptations and preferences of bacteria in response to varying pollutant levels as well as identify possible bioaccumulation patterns. This unique focus on size-dependent dynamics provides novel insights into microbial communities in polluted environments, where implications of the research could extend to the realm of bioremediation. Especially in the context of pollutant stress, certain size fractions may carry communities with enhanced ability to accumulate or metabolise mercury, providing potential bioindicators for pollution levels and unveiling potential candidates for bioremediation strategies.

KEYWORDS: size fractionation; bacterial dynamics; mercury; characterization; bioremediation

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PP68

BACTERIAL COMMUNITIES IN TYPICAL SOILS OF VOJVODINA, SERBIA

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The soil microbiome is crucial for soil health, particularly in agriculturally focused regions like the Autonomous Province of Vojvodina. Understanding the soil microbial community is key to fostering sustainable and productive farming. However, comprehensive studies on Vojvodina's soil microbiome are limited. This research aimed to analyse the microbial community composition and diversity in the three different soil types in Vojvodina: chernozem, vertisol, and solonchak. Soil was sampled across Vojvodina from May to July 2022. Environmental DNA was extracted using DNeasy PowerSoil Kit (Qiagen, Germany) and sequenced on the Illumina MiSeq platform, using primer pair 515FB-806RB for the V4 region of 16S rRNA. Sequences were analysed using QIIME2 and R Studio. Alpha diversity was measured through Observed, Chao1, Shannon, and Simpson indices. All the analysed indices were significantly different in chernozem and solonchak, while chernozem and vertisol significantly differed only in the Observed and Chao1 index. Vertisol and solonchak differed in all indices except Simpsons. Based on these indices, we conclude that diversity is high in all soil types. Principal Coordinates Analysis (PCoA) shows strong separation of soil types, meaning that significant differences in diversity amongst them exist. The most dominant bacterial phyla in each soil were Proteobacteria, Acidobacteriota, Actinobacteriota, and Verrucomicrobiota. However, differences in relative abundances across soil types were noticed. Namely, phylum Acidobacteriota was significantly more abundant in chernozem, Actinobacteriota in solonchak, and Verrucomicrobiota in vertisol. Amongst less abundant phyla, Crenarcaheota and Firmicutes were significantly more abundant in solonchak, likely due to their tolerance to high salinity present in solonchak. Community composition indicates that chernozem and vertisol are more similar based on relative abundances of phyla, compared to solonchak. In summary, this study provides a first detailed account of the bacterial community composition in Vojvodina's three typical soil types, laying the groundwork for more comprehensive future research and monitoring.

KEYWORDS: microbiome; metabarcoding; soil; 16S rRNA

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PP69

ENZYMATIC ACTIVITIES IN SOILS OF VOJVODINA, SERBIA

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Enzymatic activities serve as vital indicators of soil health, responding to minor changes in soil conditions. This is particularly relevant for agricultural areas like the Autonomous Province of Vojvodina. Our study focused on the enzymatic activity in three different soil types in Vojvodina – chernozem, solonchak, and vertisol. Soil was sampled across Vojvodina between May and July 2022 in 9 locations, with a total of 26 field plots. Two agricultural and one undisturbed soil were sampled at each location. The activities of phosphatases, dehydrogenase, and *β*-glucosidase were determined spectrophotometrically, while the activity of catalase was determined by titration method. The highest acid phosphatase activity (mean value 55.58 μg pNP·g^{-1}·dm·h^{-1}) was measured in vertisol, which was significantly higher than activity in chernozem and solonchak. Alkaline phosphatase activity was lower in all vertisol samples compared to acid phosphatase activity. Knowing that vertisol is mildly acidic soil, these results were expected. In chernozem, alkaline phosphatase activity with an average value of 36.76 μg pNP·g^{-1}·dm·h^{-1} was higher than acid phosphatase activity with an average value of 19.10 μg pNP·g^{-1}·dm·h^{-1}. The exception was plot C3, which exhibited elevated acid phosphatase activity, which is expected since this is forest soil rich in organic matter, and therefore more acidic. Similarly, alkaline phosphatase activity was generally higher in solonchak than acid phosphatase, except for plot S3, which represented the control soil. The activities of *β*-glucosidase and catalase were not found to be significantly different across the investigated soil types. Dehydrogenase activity was significantly lower in vertisol (mean value of 0.06 μg TPF·g^{-1}·dm·h^{-1}) compared to chernozem (mean value of 0.79 μg TPF·g^{-1}·dm·h^{-1}) and solonchak (mean value of 0.54 μg TPF·g^{-1}·dm·h^{-1}). These patterns of enzymatic activity reflect the unique biochemical properties of each soil type, offering insights into their respective health and fertility statuses.

KEYWORDS: microbial enzymes; agricultural soil; soil quality

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PP70

INFLUENCE OF BACTERIA LOADED BIOCHARS AND HYDROCHARS ON MICROBIAL PROPERTIES OF ALLUVIAL SEDIMENT

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Biochar and hydrochar materials embedded with microorganisms are promising for bioremediation applications. However, the impact of these amendments on microbial characteristics, essential for understanding processes like biosorption and biodegradation, remains understudied. Our study aimed to evaluate the effects of eight different *Miscanthus×giganteus* (M) and sugar beet shreds (SBS) biochars and hydrochars, each colonized with a biofilm of the organophosphorus pesticide-degrading bacterial strain *Bacillus megaterium* BD5, on the microbial properties of sediment. For this purpose, column experiments were conducted using a mix of Danube alluvial sediment and 0.5% biofilm-laden materials. We assessed bacterial counts, enzymatic activities, and community-level physiological profiles (CLPP) at the beginning and end of a 7-day experiment. The assessments were performed using the spread plate method, standard enzyme activity assays, and ECOLOG plates, respectively. Bacterial counts increased in all column experiments by 0.6 to 1.7 log units, while the lowest counts were noted in sediment without amendments. Measured enzyme activity was very low. There was no significant difference observed between the SBS and M materials, as well as between the biochars and hydrochars, in terms of bacterial counts and enzyme activity. CLPP revealed that addition of materials with BD5 biofilm significantly increased metabolic activity of microbial community compared to uninoculated control sediment. No apparent grouping of utilized substrate groups nor materials was noted based on principal component analysis. It should be noted that the drying of sediment for experimental setup likely inactivated part of the native microbial community, which may have amplified the effects of strain addition. In conclusion, *B. megaterium* BD5 adapted well to the experimental conditions, remaining viable and active. While the type of material had a negligible effect, the presence of the bacterial strain was the primary factor enhancing bacterial abundance and metabolic activity. These findings hold significant implications for biosorption and biodegradation processes within the sediment.

KEYWORDS: *Bacillus*; biochar; hidrochar; biofilm; alluvial sediment

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PP71

EXPLORING THE DYNAMICS OF FUNGAL COMMUNITIES IN POULTRY TERRA BEDDING: IMPLICATIONS FOR SOIL HEALTH AND PLANT NUTRITION

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A well-managed bedding enriched with nutrient-rich poultry manure proves to be an important organic fertilizer that improves soil health and promotes plant development. Our study looked at the complex processes of bedding formation and the associated fungal community dynamics over a forty-five day period. The bedding on day zero (SM.0) showed a significantly higher diversity of fungal genera than on the following days, which was confirmed by increased Shannon and Simpson indices. Diversity decreased noticeably on the tenth day and reached its lowest point on the twentieth day. On the forty-fifth day, the second highest alpha diversity was recorded again. Analysis of PCoA beta diversity revealed a clear separation between the bedding from day zero and the other days. In addition, the forty-fifth day bedding stood out from the tenth day bedding, confirming a clear shift in fungal communities (gradual transition from day 10 to day 45) with the onset of fermentation and decomposition processes. The bedding SM.0 showed a high diversity with *Apiotrichum* (62.41 %), *Pseudogymnoascus* (12.14 %) and *Penicillium* (5.33 %) as the most prominent. Together with *Penicillium*, these fungi play a balancing role in the soil microbial communities. On the tenth and twentieth days, similar taxa dominated, including *Diutina* (38.36% and 36.79%), *Debaryomyces* (24.36% and 18.82%) and *Trichosporon* (23.17% and 29.98%). However, on day 30, the relative abundance of *Aspergillus* increased (23.73 %). On day 45, a significant increase in *Coprinopsis* (26.55 %), *Aspergillus* (26.94 %) and *Fusarium* (6.08 %) was observed. These genera are frequently found in soil, where they play a central role in the nutrient cycle and in the decomposition of organic matter. *Coprinopsis* species in particular have been investigated for their potential use in mycoremediation of polluted sites, thus demonstrating their detoxification capabilities for the environment.

KEYWORDS: Poultry bedding; fungal communities; circular economy; soil health

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PP72

ANTIFUNGAL ACTIVITY OF *TRICHODERMA* IN THE BIOLOGICAL CONTROL OF FUNGAL PHYTOPATHOGENS

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The use of chemical fungicides harms the ecosystem by reducing the antagonistic population in the soil and pave the way for evolution of resistance in pathogens. On the other hand, *Trichoderma spp*. is an environmentally friendly plant symbiont that can serve as an alternative biocontrol agent. In this study, the antagonistic activity of six *Trichoderma spp*. (*T. citrinoviride* - 127, *T. atroviride* - MIT 3/13, *T. zeloharzianum/lentiforme* - MS/1, *T. citrinoviride* - NK 1/9, *T. citrinoviride* - X1 and *T. citrinoviride* - X2) against three plant pathogenic fungi (*Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and *Rhizoctonia sp*.) were investigated, using in vitro dual culture technique on Petri dishes (confrontation assay). The antifungal ability of *Trichoderma spp*. isolates was studied by Radial Growth Inhibition factor (RGI). *Trichoderma zeloharzianum/lentiforme* (MS/1) showed the highest antifungal activity against all phytopathogens tested. The *Trichoderma* MS/1 strain completely stopped the growth of *Sclerotinia sclerotiorum* and *Rhizoctonia sp*., (RGI 100 %) and reduced the growth of *Sclerotium rolfsii* (RGI 91 %). *Sclerotinia sclerotiorum* was the most susceptible phytopathogen strain among the *Trichoderma* isolates tested (RGI between 90 and 100 %), while *Rhizoctonia sp*. and *Sclerotium rolfsii* were slightly less suppressed in the confrontation assay (RGI between 82 and 100 %). Overall, our preliminary results showed that all tested Trichoderma isolates possess a considerable antifungal/antagonistic effect against the investigated phytopathogens and thus proved to be efficient biocontrol agents.

KEYWORDS: *Trichoderma*; biological control agents; plant pathogens; confrontation assay

ACKNOWLEDGEMENT: This research was supported by the Science Fund of the Republic of Serbia, #grant no 4545, Advanced Biophysical methods for soil targeted fungi based biocontrol agents - BioPhysFUN.

PP73

POTENTIAL ENVIRONMENTAL TOOL FOR THE BIOREMEDIATION OF CONTAMINATED SOILS

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The use of microorganisms in the bioremediation of soils treated with pesticides and after an accidental oil spill is one of the most environmentally friendly approaches compared to physico-chemical treatments, which often lead to disruption of soil ecosystem function. The greatest attention should be paid to agricultural land, as pesticides and petroleum hydrocarbons have the property of bioconcentration and bioaccumulation and easily enter the food chain. The toxic effect of these widely used organic chemicals can have various effects on the health of living organisms, including humans. Bioaugmentation, which is based on the introduction of active microorganisms into the polluted area, is used to enhance the biodegradation of organic pollutants. Bioaugmentation requires the selection of the most active microorganisms that have a high potential for targeted degradation of the organic matter in question and the development of a technology for their use that takes into account the complexity of the soil characteristics and the degree of pollution. The ability of microorganisms to metabolize different xenobiotics is due to the content of different enzymes that play an important role in the basic phases of biodegradation. In this work, the isolation, identification and characterization of autochthonous microbial cultures from soil samples contaminated with the herbicide pendimethalin and crude oil were performed. Morphology, biochemical tests and MALDI TOF analysis were used to identify the most common bacterial cultures in the soil samples analysed. The potential for biodegradation of pendimethalin and crude oil is shown by isolates from the genera *Bacillus* and *Pseudomonas*. The activity of microbes is very important for the restoration of our environment. It is necessary to support the activities of indigenous microorganisms in polluted soils and improve their biodegradability through bioaugmentation.

KEYWORDS: bioremediation; soil; pendimethalin; crude oil; bioaugmentation

PP74

GROWTH OF RHIZOBIAL STRAINS AND THEIR PLANT GROWTH PROMOTING ACTIVITY UNDER HEAVY METAL STRESS

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Heavy metals increase in the soils has become a worldwide issue. The growth and nutrient balance of different legumes including alfalfa could be disrupted in soils with increased nickel concentrations. Inoculation of legumes with rhizobia, nitrogen-fixing bacteria, can decrease concentrations of heavy metals in plants and could be used for phytostabilization of heavy-metal contaminated soils. The mechanisms of rhizobia for plant growth promotion partly overlap with protection mechanisms used by metal-tolerant rhizobacteria. Besides nitrogen fixation, some of them are the producers of plant hormones, which promote plant growth; such is indole-3 acetic acid. Therefore, the purpose of this study was to select nickel tolerant rhizobial strains and evaluate their indole-3 acetic acid production under nickel stress. Screening alfalfa rhizobial strains for nickel tolerance showed that all of the tested strains could grow well in the medium supplemented with 0.1 mM Ni, and some of the tested strains could tolerate up to 1.1 mM Ni, that is the highest Ni concentration tested in this study. Generally, the addition of Ni in the range from 0.05 to 0.4 mM affected the production of IAA in the medium supplemented with tryptophan, as a precursor for IAA, but the IAA levels still remained very high. The IAA was not detected at 0.7 mM Ni. The alfalfa rhizobial isolates are overall relatively sensitive to Ni concentrations. Some strains with high nitrogen fixation capacity and IAA production were sensitive to Ni, indicating the importance of metal tolerance evaluation. The selected IAA producing Ni tolerant rhizobial strains are potential candidates for further investigation in nickel biosorption assays and heavy metal stress alleviation in plants.

KEYWORDS: alfalfa rhizobia; heavy metals; plant growth promotion

ACKNOWLEDGEMENT: This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 7015, Utilizing rhizobia to reduce the risk of heavy metal accumulation in alfalfa: Nickel (Ni) case study - RhizoDETOX.

PP75

ANTIBIOTIC RESISTANCE OF GENUS *VIBRIO* IN FISH AND BIVALVE AQUACULTURE: CASE STUDY OF ADRIATIC SEA

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Aquaculture is rapidly expanding globally, particularly in marine environments. In Croatia, mariculture involves mainly seabass and seabream farming and cultivation of bivalves. Due to its reliance on natural environments, aquaculture is vulnerable to environmental changes and disease outbreaks. *Vibrio* bacteria, one of the most common bacteria in marine environments, cause vibriosis, a prevalent bacterial disease in the Adriatic region affecting both fish and bivalve aquaculture. Certain *Vibrio* species, like *V. harveyi* and *V. alginolyticus*, are associated with mass mortalities in both fish and bivalve aquaculture. Furthermore, human pathogens, *V. alginolyticus*, *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus*, infect humans through consumption of contaminated seafood or by exposure through open wounds. The emergence and spread of antibiotic resistance related to excessive anthropogenic pressures on marine environments is of public health concern worldwide but is poorly studied in the eastern Adriatic Sea aquaculture. Our study aimed to determine antibiotic resistance profiles of *Vibrio* species in three fish aquaculture regions (North, Central, and South Adriatic) and two bivalve aquaculture regions (North and South Adriatic) and compare differences between regions and aquaculture types. Most prevalent species in both aquacultures and all sites where from Splendidus and Harveyi clade. The results show definitive differences between the three regions in fish aquaculture for some antibiotics, while for bivalve aquaculture, differences between regions have not been recorded. Furthermore, we found distinctive differences in antibiotic resistance profiles between different *Vibrio* clades. These results indicate that there are differences among regions (for fish aquaculture), which suggest additional pressures on these regions. Additionally, the occurrence of antibiotic resistance in bivalve aquaculture indicates other pathways for antibiotic entry into marine environments since antibiotics are not used in bivalve farming.

KEYWORDS: *Vibrio* bacteria; aquaculture; bivalve; fish; Croatia

ACKNOWLEDGEMENT: Results are collected as part of project AQUAHEALTH (HRZZ IP-2014-09-3494), AqADAPT (HRZZ IP-2018-01-3150), BEST ADRIA (HRZZ IP-2019-04-1956) and Career Development of Young Researchers (grant No DOK 2021-02-7567) funded by Croatian Science Foundation.

PP76

GENOMICS INFORMED DESIGN OF NOVEL ASSAY FOR HIGHLY SPECIFIC DETECTION OF *XANTHOMONAS ARBORICOLA* PV. *CORYLINA*

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The bacterial blight, caused by Xanthomonas arboricola pv. corylina (Xac), is a re-emerging disease, severely affecting the productivity of hazelnut production worldwide. Due to lack of timely and reliable detection of the pathogen in plant material, as well as identification of the bacterium, the aim of this work was to develop and validate Xac- specific molecular methods. In silico comparative analysis of six Xac genomes available in genomic databases was used for detection of a highly specific molecular markers and primer design for conventional PCR, Real-time PCR (SYBR® Green and TaqMan™) and LAMP-PCR. Primer specificity was studied by in vitro analysis of a collection of Xac strains (n = 60) and other related species (n = 30). The sensitivity of the methods was studied using serial dilutions of genomic DNA, as well as hazelnut plant tissue extract. A specific region of 2,440 bp, located on the chromosome, was selected for design of several primer pairs for each method, which resulted in amplification of specific sequences in all Xac strains, while in other tested bacterial strains the result was negative. The sensitivity of the reaction and the detection threshold varied depending on the method, the primer set, and the type of material used for the pathogen detection. The development and implementation of new detection system for the causative agent of hazelnut bacterial blight represents a significant advance in the diagnosis of this pathogen. The advantages of the Real-time and LAMP-PCR methods are monitoring of the results in real time, while the advantages of the LAMP-PCR are simplicity and possibility of the method application outside the laboratory.

KEYWORDS: hazelnut; diagnosis; PCR; LAMP; qPCR

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PP77

DEGRADATION OF POLYAMIDE/POLYURETHANE TEXTILE BLEND BY *STREPTOMYCES* SP. R1

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The increasing production and utilization of synthetic polymers in the textile industry over the past five decades has raised concerns about the environmental impact of the industry. The recalcitrant nature of synthetic fibers hampers the biodegradation of these textiles in the environment and leads to the accumulation of textile waste. Effective solutions for recycling and proper disposal of textile waste are lacking, however, the use of microorganisms and enzymes has emerged as a promising approach. The genus *Streptomyces* has been well studied as a producer of different hydrolytic enzymes, several of which have found use in industrial settings as well. As an integral part of the soil microbiome, *Streptomyces* species have been shown to interact with different textile materials in soil and may play a role in the degradation of these materials. This study aimed to examine the interaction of *Streptomyces* sp. R1, isolated from the rhizosphere of *Cotinus coggygria*, with polyamide/polyurethane textile, and identify potential enzymes involved in the biodegradation of synthetic textiles. The degradation of the textile was tested in liquid cultures (minimal salt medium) and model compost, bio-augmented with *Streptomyces* sp. R1 for 4 months. After the incubation, morphological, and changes in the functional groups of the textiles were analysed using scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). The surface of the textile showed noticeable cracks and fissures after 4 months of burial in the bioaugmented model compost, alongside changes in the functional groups of the polyamide/polyurethane textile, which indicates biodegradation of the synthetic fibers. Searching the genome of *Streptomyces* sp. R1, several enzymes involved in the degradation of synthetic polymers were identified, including an esterase homologous to highly efficient plastic degrading depolymerases. Overall, the results presented here indicate *Streptomyces* sp. R1 has the potential for synthetic textile degradation and bioremediation.

KEYWORDS: *Streptomycetes*; biodegradation; textile; synthetic polymers; composting

MICROBIAL GENETICS, METAGENOMICS, AND METAPROTEOMICS

IL78

NEXT-GENERATION SEQUENCING AND BIOINFORMATICS: HOW DOES MICROBIOLOGY BENEFIT FROM CUTTING EDGE TECHNOLOGIES?

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It was 20 years ago that the first high throughput sequencing technology was released on the market, this event started the Next-Generation Sequencing (NGS) revolution. The advent of NGS reshaped various domains of Life Sciences, including microbiology, by facilitating high-throughput and cost-effective analysis of microbial genomes and communities, and led to the discovery of previously unknown microbial taxa. The resequencing of both model and non-model bacteria challenged the traditional views of bacterial species and boosted the emergence of the concept of pan-genomics, providing a more comprehensive understanding of genomic diversity and phenotypic plasticity. Moreover, the application of NGS to the field of metagenomics has greatly improved our understanding of microbial ecology, shedding light on the microbial "dark matter" and its vital role in the ecosystem processes. The wealth of data allowed a more in-depth study of the low-abundance fraction of the microbiome in well-known microbiomes such as the one present in the rhizosphere and in fermented food. On the other hand, it made possible the exploration of previously unexplored environments such as groundwater and endolithic communities. Despite these remarkable advancements, there are still some challenges to be overcome, regarding the development of *ad-hoc* pipelines, the standardization of data management procedures, the integration of data deriving from different ´omics datasets and the validation *in-vivo* of the findings derived from the bioinformatic analyses. Addressing these challenges will be crucial in order to exploit the full potential of NGS in microbiological research.

KEYWORDS: next-generation sequencing; bioinformatics; microbial genomics; metagenomics; microbial ecology

IL79

INSIGHTS INTO THE EVOLUTION OF MULTIPARTITE GENOMES IN PROTEOBACTERIA

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Multipartite genomes, consisting of more than one replicon, have been found in approximately 10% of bacteria, many of which belong to the Proteobacteria. Many aspects of their origin, evolution, and the possible advantages related to this type of genome structure remain to be elucidated. Through a combination of phylogenetic and pangenome-level functional enrichment analyses, we determined the presence and distribution of multipartite genomes in different class of Proteobacteria, to clarify some aspects of their origin and the possible advantages associated with them. Our data suggest that the emergence of secondary replicons in *Proteobacteria* is rare and that they derived from plasmids. Despite their origins, we highlighted some evolutionary trends such as the inverse proportionality of the genome to chromosome size ratio. We also highlighted some functional trends. The core gene set of the secondary replicons is extremely small, probably limited to essential genes or genes that favour their maintenance in the genome. This hypothesis agrees with the idea that the primary advantage of secondary replicons could be to facilitate gene acquisition through HGT, resulting in replicons enriched in genes associated with adaptation to different ecological niches. Indeed, they are enriched both in genes that could promote adaptation to harsh environments, and in functional categories related to the exploitation of environmental resources, which can complement chromosomal functions.

KEYWORDS: multipartite genomes; genome evolution; chromids; secondary replicons; proteobacteria

IL80

GENOME LEVEL INVESTIGATION OF INTER-KINGDOM MICROBIAL INTERACTIONS

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Past empirical research has demonstrated that bacterial interaction might enhance algal biomass production and algal biohydrogen evolution. To investigate the mechanisms and microalgal functions activated under bacterial associations, different bacterial species were co-cultivated with *Chlamydomonas reinhardtii* cc124 green algae. Bacterial species were isolated from diverse environments including biogas sludge, soil and commercial agricultural biostimulant products. Pairwise algal-bacterial combinations were cultivated for five days in synthetic wastewater. Successful bacterial candidates were identified by high specific algal biohydrogen production and increased specific algal biomass. We have investigated the effects of bacterial phylogenetic relationship and growth rate on algal growth, nutrient intake and biohydrogen production. Anaerobic digestion (AD) is a microbe-driven process of biomass decomposition, which is an environmentally friendly model of bio-waste valorization and nutrient recycling. Microbial indicators of optimal performance and benchmark values for well-performing reactors help monitoring sustainable operation of the AD process. We have examined the microbial community of three Hungarian state-of-the-art industrial biogas plants utilizing distinct biomass compositions as main substrates. Cutting-edge deep neural network-guided genome resolved metagenomics approach was used to reveal the interactions among the members of the anaerobic microbial „dark matter". Metagenome and metatranscriptome data showed a stable core microbiome in the digesters, predominated by biopolymer decomposers and syntrophic bacteria being in a strong interaction with hydrogenotrophic methanogenic archaea.

KEYWORDS: algal-bacterial interactions; anaerobic digestion; genome centric metagenomics

IL81

ROLE OF THE STRINGENT RESPONSE IN *PSEUDOMONAS AERUGINOSA* VIRULENCE

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The opportunistic human pathogen *Pseudomonas aeruginosa* is one of the most threatening Gram-negative pathogens for which new antibiotics are urgently needed. It causes a variety of hard-to-treat infections, including lethal lung infections in people with cystic fibrosis, a genetic disease affecting 1/3000 newborns/year. The ability of *P. aeruginosa* to resist to adverse environmental conditions and colonize multiple environments, including plant and animal tissues, mainly relies on its dynamic network of regulatory circuits, which control the adaptive response to environmental cues. Stringent response (SR) is a widely conserved bacterial global regulatory pathway controlling survival strategies in stressing environments, including infection sites. In Gram-negative bacteria, SR relies on the combined action of the (p)ppGpp second messenger and the DksA protein. *P. aeruginosa* is unique as it expresses two functional DksA paralogs: DksA1 and DksA2. Using RNA-seq and phenotypic assays, we showed that DksA1 and DksA2 are eco-paralogs with indistinguishable function but optimal activity under different environmental conditions, highlighting their mutual contribution to *P. aeruginosa* virulence. SR also plays an important role in *P. aeruginosa* intracellular life. Indeed, despite *P. aeruginosa* is an extracellular pathogen, it is able to persist within and to kill specialized and not specialized phagocytic cells. Intracellular survival of *P. aeruginosa* could be an important strategy to evade the host immune system and to escape antibiotic activity, especially in chronic infections. Finally, SR is hierarchically dominant over two major *P. aeruginosa* regulatory circuits, *i.e.,* quorum sensing and cyclic-di-GMP signalling systems, supporting this regulatory system as a promising target for the development of new antimicrobials.

KEYWORDS: *Pseudomonas aeruginosa*; stringent response; virulence; DksA; global gene regulation

IL82

THE ROLE OF GUT MICROBIOTA ON INFECTION WITH MULTIRESISTANT BACTERIA

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Any microbiota on human body can be a reservoir of multi-drug resistant bacteria and resistance genes, but most research has been done on the gut microbiota. Intestinal microbiota is a complex population of microorganisms that perform many tasks, one of the most important of which is colonization resistance. In healthy microbiota the numbers of multidrug resistant bacteria are low, and colonization is transient. Disrupted microbiota and high exposure to resistant bacteria results in higher colonization, mainly with Enterobacteriaceae and enterococci. Further consequences of dysbiotic microbiota are the increased frequency of horizontal gene transfer and thus the increase in the number and diversity of resistance genes in the gut; the increased gut permeability and increased potential for systemic spread of multidrug-resistant bacteria; and changes in systemic immunity and potentially more severe extraintestinal infections.

Recently, new microbiota-targeted strategies are in development to reduce colonization with resistant bacteria. These include fecal transplantation, probiotics, prebiotics, and microbiota protection approaches and can be used in both the treatment and prevention of infections.

KEYWORDS: microbiota; colonization resistance; dysbiosis; fecal transplantation; probiotics

IL83

DANGEROUS RELATIONS: BACTERIA, ANTIMICROBIAL THERAPIES, AND ALLERGIC DISEASES

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In the last few decades, the incidence and prevalence of allergic disorders have increased progressively throughout the world. Allergic diseases are listed by the World Health Organization (WHO) as one of the top three disorders to be prevented and controlled in the 21st century. The increased incidence of allergic disorders may be the result of a relative fall in microbial induction in the intestinal immune system during infancy and early childhood. Microbiota composition and associated metabolic activities are essential for the education and development of a healthy immune system. Microbial dysbiosis, caused by risk factors such as diet, birth mode, or early infant antimicrobial therapy, is associated with the inception of allergic diseases. In turn, allergic diseases increase the risk for irrational use of antimicrobial therapy. Microbial therapies, such as pre-, pro-, or postbiotics, have been studied in the prevention and treatment of allergic diseases, but evidence remains limited due to studies with high heterogeneity, strain-dependent effectiveness, and variable outcome measures.

KEYWORDS: allergic disease; bacteria; children; antimicrobial therapy; microbiota

IL84

MODULATION OF HUMAN MYCOBIOTA AS A TOOL FOR PROMOTING HEALTH

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Human microbiota is a complex microbial ecosystem that colonizes the human body. The densest microbial ecosystem is present in the colon, which can be seen as a bioreactor, while colonic microbiota can be perceived as a microbial organ of the human body. Colonic microbiota performs numerous metabolic functions that complement human metabolism and systemically impact health. In this complex ecosystem, bacteria are the dominant fraction but other types of organisms belonging to archea, fungi (including yeast) and protozoa are present too. Recent data indicates that colonic fungi (i.e. mycobiota) are a sub-abundant but highly relevant component of the microbiota. The bacterial and fungal compositions are strongly interconnected and in several intestinal diseases an abnormal mycobiota composition along with bacterial dysbiosis was detected. The most prevalent and the most dominant genera of human mycobiota are *Saccharomyces*, *Malassezia*, *Candida*, *Cyberlindnera*, *Cladosporium*, *Debaryomyces* and *Pichia*. Dysbiosis of mycobiota was detected in celiac disease, inflammatory bowel disease and hepatocellular carcinoma, while an experiment on a mouse model showed that mycobiota dysbiosis can promote colon cancer by changing metabolism of subepithelial macrophages. Mycobiota is relatively unstable and, presumably, strongly influenced by the diet and other environmental factors. Therefore, for treatment of mycobiota dysbiosis, in addition to antifungal drugs, dietary supplements can be used. Polyphenols are bioactive plant metabolites, which are classified as prebiotics. These molecules have the ability to promote the growth of beneficial and inhibit the growth of pathogenic microorganisms. While this feature has been long known for bacteria, in recent studies we show that several medicinal plant polyphenols can stimulate the growth of the probiotic yeast *Saccharomyces boularii*, and suppress the growth of the opportunistic pathogen *C. albicans*. Based on this preliminary in vitro data, treatments with traditional medicinal plants might offer a pave a way to alternative approach to currently incurable diseases.

KEYWORDS: microbiota; polyphenols; *Candida*; *Saccharomyces*; medicinal plants

IL85

MICROBIOMES IN ORAL CARCINOMAS

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Oral cancer, particularly oral squamous cell carcinoma (OSCC), imposes a substantial global health burden, characterized by fast metastasis, high recurrence rates, and drug resistance, with over 350,000 cases and ~170,000 deaths annually. In addition to well established risk factors like tobacco and HPV, the intricate interplay within the oral microbiome, particularly its dysbiosis, has been implicated in OSCC development. Moreover, emerging evidence suggests that systemic and distant microbiomes may also play a contributory role. This broader perspective takes into account the interconnectedness of various microbial communities in the body, in particular within the "oral-gut-systemic axis", facilitated by bacterial translocation and immune signalling, and their potential influence on oral cancer initiation and progression. Understanding the crosstalk between these microbiomes and their potential influence on oral carcinogenesis represents a novel and evolving aspect in this research domain. Potential mechanisms through which specific bacterial species or overall dysbiosis contribute to OSCC include generation of harmful metabolites and byproducts, sustaining an inflammatory environment that promotes tumorigenesis, modulation of anti-tumour immune response and facilitation of tumour growth and promotion of cancer invasion and dissemination. Further research is needed to unravel the specific mechanisms by which oral and non-oral microbiomes may contribute to oral cancer. A deeper comprehension holds promise for identifying novel diagnostic markers, developing innovative preventive strategies, clinical monitoring protocol and therapeutic interventions. The integration of multi-site microbiome analyses may provide a more holistic understanding of the complex interactions involved in the initiation and progression of oral cancer.

KEYWORDS: oral cancer; microbiomes; dysbiosis; carcinogens; immunomodulation

ACKNOWLEDGEMENT: Supported by the Science Fund of the Republic of Serbia, #GRANT No 7750038, Oral Cancer – New Approaches in Prevention, Control and Post-Operative Regeneration – An *In Vitro* Study – ORCA-PCR.

IL86

THE USE OF INTEGRATIVE MULTI-OMICS APPROACH IN CULTIVATION AND CHARACTERIZATION OF GUT BACTERIA RELATED TO MICROBIOTA-GUT-BRAIN AXIS AS A SOURCE FOR NEXT GENERATION PROBIOTICS

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There has been an epidemic of various non-communicable degenerative and autoimmune diseases, strongly associated with the modern lifestyle. Among them, neurodegenerative and psychiatric disorders represent a huge burden on society. Recently, all these diseases have been associated with the gut microbiota dysbiosis. Gut microbiota-host interaction research has been greatly improved due to development of molecular high-throughput techniques based on various 'omics' techniques coupled with bioinformatics and data science developments. However, the mechanisms of the host–microbiota cross-talk are still poorly understood. The NextGenBiotics project proposes an innovative integrative multi-omics research strategy for deciphering the mechanism behind the cross-talk among microbiota and gut-brain-axis. The 118 novel NGPs candidates belonging to *Dorea* sp., *Blautia* sp., *Bacteroides* sp., *Roseburia* sp., *Sellimonas* sp., *Faecalicatena sp., Phascolarctobacterium faecium*, and *Faecalimonas sp.* were cultivated. The 25 NGPs with confirmed safe status and potential probiotic potential were screened in *C. elegans* model for their effects on behavioural and neuronal activity. The most prominent candidates with ability to upregulate expression of genes involved in neurotransmiting are further tested in EAE (an animal model for MS) and CUMS depression model. The specific microbiota-derived metabolites have been identified as potential neuro- and psycho-biotics. The NextGenBiotics is highly ambitious project, dedicated to pioneering work in the field of multi-omics studies related to the cultivation of novel anaerobic NGPs and the studying of their effect on MGBA. This concept enabled studying bidirectional communication between gut microbiota and brain on the functional level that will significantly contribute to the growing body data related to MGBA. The results obtained during NextGenBiotics determined the genes/metabolites and the associated mechanisms involved in health-promoting effects of NGPs in MGBA beyond state-of-the-art, broadening the scientific knowledge and opening up the possible novel therapeutic approaches in prevention and therapy of neurodegenerative and psychiatric diseases.

KEYWORDS: next generation probiotics; microbiota-gut-brain axis; neurobiotics; psyhobiotics

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IL87

CHALLENGES IN METAGENOMIC ANNOTATION OF ANTIBIOTIC RESISTOME

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The antibiotic resistance genes (ARGs) in both host-associated and environmental microbiomes – antibiotic resistome – play an important role in the spread of antibiotic resistance. Metagenomics enables high-throughput exploration of microbiomes. One important step in these analyses is ARGs annotation within the metagenomes, for which several tools are in use. However, most of these tools are developed for genomics studies and their databases may pose certain biases. We aimed to compare outputs from different ARGs annotation tools for metagenomes and detect their potential challenges in microbiome studies. We ran >13 000 high-quality metagenomes from 14 habitats (Coelho et al., 2022; https://gmgc.embl.de/) through three ARGs annotation pipelines (with default settings) using DeepARG (CARD, ARDB, UNIPROT; https://bench.cs.vt.edu/deeparg), RGI (CARD; https://card.mcmaster.ca/) and ABRicate (CARD, ResFinder, ResFinderFG, NCBI, MEGARes, ARG-ANNOT; https://github.com/tseemann/abricate). To facilitate comparison of outputs with different gene names, we performed ARO normalization using a novel tool https://github.com/BigDataBiology/argNorm i.e. mapping ARG annotation databases to CARD's antibiotic resistance ontology. DeepARG and RGI provide higher coverage (with potential novel ARGs and/or false positives) while ABRicate with different databases has lower coverage but more well validated ARGs. The annotations did not differ only in number of hits but also in the information provided: e.g. using ABRicate, more sulfonamide resistance genes were identified using ResFinderFG as the database (ARGs obtained by functional metagenomics by Gschwind et al., 2022), while using ResFinder resulted in more macrolide-resistance genes. Thus, choice of annotation tool and database should be driven by research questions and ARGs targets.

KEYWORDS: microbiome; metagenome; antibiotic resistance gene

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IL88

PLANT MICROBIOMES: FROM DIVERSITY TO HEALTHY CROPS

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Plant microbiomes are important bioresources for agriculture, as beneficial microbes can improve plant health and yield by inhibiting phytopathogens, promoting plant growth, enhancing nutrient uptake, etc. With the intensification of pesticide use, the balance of plant-associated microbial communities is being dramatically disrupted. In view of the growing interest in safe and nutritious food, biofertilizers and biopesticides are promising alternatives for modern sustainable agriculture. Biological control of plant diseases is not only an alternative to chemical pesticides but can also enable the control of diseases that cannot be controlled with other control strategies. Our aim is therefore to characterise environmentally compatible microbial inoculants to find a sustainable way for the health management of agroecosystems. The diversity of microbial communities associated with different crops was studied using traditional culture-dependent methods as well as next-generation sequencing techniques. *Bacillus thuringiensis*, *Pseudomonas* sp. and *P. synxantha* strains isolated from the phyllosphere and from crown gall tumour were selected for their antagonistic activity and further tested for their biocontrol potential in planta. In addition, a complete analysis of the phytobiome of the treated and non-treated plants was performed. Finally, whole genome sequencing of a few most promising antagonistic strains was performed. The results confirmed the hypothesis that autochthonous strains derived from diseased plants are the best source of potential biocontrol agents. Metabarcoding analysis revealed that the application of beneficial strains did not affect the structure of the microbiome. Our research expands the knowledge of the plant microbiome and its potential to improve plant health. By characterising indigenous beneficial strains, we contribute to sustainable agriculture and reduce the environmental impact of synthetic pesticides.

KEYWORDS: beneficial bacteria; biocontrol; phytopathogens; DNA metabarcoding; sustainable agriculture

ACKNOWLEDGEMENT: This research was supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia and Science Fund of the Republic of Serbia through Serbian Science and Diaspora Collaboration Program: Knowledge Exchange Vouchers (Contracts Nos. 451-03-66/2024-03/200178, BIOCTA 6436066).

IL89

THE IMPORTANCE OF PROBIOTICS AND MODULATION OF MICROBIOTA IN GASTROENTEROLOGY

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More than 200 billion microorganisms, including bacteria, viruses, and fungi, are thought to inhabit our gastrointestinal tract, forming the gut microbiota. Under regular conditions, microorganisms live in eubiosis and "peacefully" co-exist. Gut microbiota plays an important regulatory role, both on the intestinal level and by exhibiting systemic effects. The gut microbiota prevents pathogen colonization, regulates intestinal immunity, provides essential nutrients and bioactive metabolites. The stability and diversity of the gut microbiota are of key importance for its optimal functioning. However, under conditions of disruption of said equilibrium, which may be caused by certain external and/or internal factors, dysbiosis occurs. Dysbiosis paves the way to various diseases, such as irritable bowel syndrome, inflammatory bowel disease, and Helicobacter pylori gastritis. However, a healthy microbiome is also essential in alleviating or managing various diseases, including metabolic disease, cardiovascular and neurological disorders, and tumor development. Gut microbiota modulation has emerged as a promising tool for enhancing human health and disease prevention and treatment. Probiotics play a crucial role in gut microbiota modulation. These microorganisms can enhance gut barrier function, stimulate immune responses, and inhibit the growth of pathogenic bacteria, thereby promoting intestinal homeostasis. Additionally, probiotics can produce bioactive metabolites such as short-chain fatty acids, which exert various physiological effects on the host. It has been shown that probiotics exhibit good prophylactic, ameliorative, and curative effects in health and disease management. However, beneficial effects of probiotics seem to be strain-specific. Considering that one size really doesn't fit all, careful probiotic selection and implementation are warranted in order to achieve an optimal outcome. Nonetheless, probiotics have certainly become the most popular interventions targeting the gut microbiome and its metabolites in clinical settings.

KEYWORDS: gut microbiota; modulation; probiotics

OF90

DESCRIPTION OF A NEW POTENTIAL AGGREGATION FACTOR FROM THE *STREPTOCOCCUS THERMOPHILUS* GENOME

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Autoaggregation, the ability to self-aggregate, is widespread among both Gram-positive and Gram-negative bacteria. The functional role of aggregation is not fully understood, but it is believed to be involved in the adaptation of bacteria to environmental conditions (PMID: 31294207). One interesting class of compounds responsible for the aggregation of lactic acid bacteria is aggregation factors—surface high-molecular-weight proteins rich in threonine and lysine (PMID: 30027759). Recently, our research group discovered a new strain of *Streptococcus thermophilus* in the mountainous regions of Serbia, exhibiting an aggregation phenotype. Aggregation phenotype was confirmed visually and using microscopy. Complete genome of Agg+ strain was sequenced using NGS and a gene encoding a potential aggregation factor, which was named *aggS* was identified. The predicted threonine (12.5%) and lysine (10.5%) rich protein contains 2367 amino acids, with an average molecular weight of 255986.63 Da. AggS also contains two cysteine residues, whereas previously well-described aggregation factors of this type did not contain any cysteine residues. The predicted protein includes an N-terminal YSIRK-like signal sequence and an LPXTG cell wall anchor domain. It has 6 Mucin binding domain repeats alternating with 6 Mub B2-like domain repeats. Additionally, we found a region resembling an ice-binding domain. Given that these bacteria endure prolonged periods of low temperatures, it can be speculated that this surface membrane protein also helps the bacteria withstand freezing. The fact that the alignment using BLASTp revealed AggS to be most closely related to an uncharacterised protein from the genome of *Lactococcus garvieae*, along with the discovery of a transposase gene sequence upstream of the gene, suggests that the aggregation factor was likely acquired through horizontal gene transfer. We plan to clone it into a shuttle vector and investigate the aggregation phenotype using a heterologous expression system in *Lactococcus lactis*, as well as explore its other functions.

KEYWORDS: aggregation; NGS analysis; *Lactobacillales*

OF91

FROM GUT TO LAB: UNLOCKING ANTI-INFLAMMATORY POTENTIAL WITH GABA-PRODUCING BACTERIA

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Psychobiotics are live bacterial strains impacting the central nervous system, producing neuroactive substances like GABA. GABA from microbiota influences neural signals, affecting neurological parameters, sleep, appetite, mood, and cognition, traversing the intestinal barrier to bind to receptors on enteric neurons and the vagus nerve. *Lactobacillus* and *Bifidobacterium* species can synthesize GABA from dietary glutamate, with *Lactobacillus rhamnosus* shown to reduce anxiety and depressive behavior, elevating hippocampal GABA. Limited knowledge exists about anaerobic GABA producers, warranting further research for a comprehensive understanding. Material for isolation comprised fecal samples from healthy donors, with isolation conducted in an anaerobic chamber within a maximum of 1 hour after sampling. Isolated bacteria were identified through sequencing the 16S rRNA gene. For bacterial cultivation, different types of media were used. PYG medium contains hematine and vitamin K, essential supplements for the cultivation of anaerobic bacteria. All media included 0.1% L-cysteine, playing a role in oxygen reduction, and 0.5% glutamate, a precursor for GABA production. After identification, the presence of GABA in 8 tested bacterial species was determined using the TLC method. Quantification of GABA was performed using the HPLC method. Furthermore, the positive effects observed in Caco2 cells with induced inflammation, after treatment with certain anaerobic postbiotics producing GABA, indicate the potential anti-inflammatory effects of these postbiotics. The study implies anti-inflammatory effects of anaerobic GABA producers, offering insights into the complex interplay among gut microbiota, immune function, and mental health. Recognizing inflammation's role in depressive symptoms, targeting anaerobic bacteria involved in GABA synthesis could modulate neurotransmitters and inflammatory responses, presenting a comprehensive approach to mental well-being. Advancing research in this area contributes to a holistic understanding of anaerobic bacteria, GABA production, gut microbiota, and mental health. This offers avenues for novel therapeutic approaches and enhances overall quality of life.

KEYWORDS: GUT bacteria; GABA; psychobiotics; anti-inflammatory potential

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OF92

HUMAN CYTOMEGALOVIRUS ONCOPROTECTION ACROSS DIVERSE POPULATIONS, TUMOUR HISTOLOGIES, AND AGE GROUPS: THE RELEVANCE FOR PROSPECTIVE VACCINAL THERAPY

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The oncogenicity of the human cytomegalovirus (CMV) is currently being widely debated. Most recently, mounting clinical evidence suggests an anti-cancer effect via CMV-induced T cell-mediated tumor destruction. However, data mostly come from single-center studies and *in vitro* experiments. Broad geographic coverage is required to offer a global perspective. Our study examined the correlation between country-specific CMV seroprevalence (across 73 countries) and the age-standardized incidence rate of 34 invasive tumors. The populations studied were stratified according to decadal age periods as immunologic effects of CMV seropositivity may depend on age at initial infection. The International Agency for Research on Cancer of the World Health Organization (IARC WHO) database was used. Multivariate linear regression analysis revealed a worldwide inverse correlation between CMV seroprevalence and incidences of 62.8% tumors. Notably, this inverse link persists for all cancers combined (Spearman's $\rho = -0.732$, $p < 0.001$; $\beta = -0.482$, $p < 0.001$, adjusted $R^2 = 0.737$). An antithetical and significant correlation is also observed in particular age groups for the vast majority of tumors. Our results corroborate the conclusions of previous studies and indicate that this oncopreventive phenomenon holds true on a global scale. It applies to a wide spectrum of cancer histologies, additionally supporting the idea of a common underlying mechanism – CMV-stimulated T cell tumor targeting. Although these results further advance the notion of CMV-based therapies, in-depth investigation of host-virus interactions is still warranted.

KEYWORDS: cytomegalovirus; oncogenesis; oncoprotection; cancer; global; T cell

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OF93

EXPLORING *E. COLI*-BASED EXPRESSION OF GENETICALLY INACTIVATED TETANUS TOXIN FOR VACCINE DEVELOPMENT

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Tetanus toxin, a highly potent neurotoxin produced by *Clostridium tetani*, is the primary agent responsible for causing tetanus. This serious, potentially fatal disease can be effectively prevented through vaccination. Thanks to successful vaccination campaigns, tetanus has become exceedingly rare in both developed and most developing countries. However, the widespread presence of *C. tetani* spores in the environment means that tetanus cannot be completely eradicated, underscoring the ongoing need for vaccination. Traditionally, tetanus vaccines are produced by cultivating *C. tetani*, extracting a crude form of the tetanus toxin, and then chemically inactivating it for use in immunization. This method has proven clinically effective and is in widespread use. A challenge with this approach, however, is that the vaccine contains hundreds of various *C. tetani* proteins, with the active component making up only a variable and small fraction of the overall vaccine mass. To improve the current tetanus vaccine, there is potential in the recombinant production of a genetically inactivated tetanus vaccine. Prior studies have demonstrated the feasibility of engineering the full-length tetanus toxin in *E. coli*, and our current work builds on this foundation. We have successfully cloned the complete tetanus toxin open reading frame into the pMAL expression vector. This step was followed by the creation of a genetically inactivated protein, achieved through standard site-directed mutagenesis which altered 8 critical amino acid residues. These mutations have been confirmed via sequencing, ensuring that the toxin is genetically inactivated and thus does not require chemical inactivation for vaccine production. Our present focus is on optimizing the expression of this protein in *E. coli*. Following this, we intend to conduct thorough assessments of the biochemical and immunological properties of the recombinant tetanus toxin. This research represents a promising avenue towards enhancing the efficacy and specificity of tetanus vaccines, potentially improving global health outcomes.

KEYWORDS: tetanus toxin; genetic inactivation; site-directed mutagenesis; vaccine; biotechnology

OF94

MICROBIOME ASSOCIATED WITH MYCELIAL ACTIVITY OF THREE SPECIES OF BASIDIOMYCETES: FAIRY RINGS IN THE GARDENS OF THE ROYAL PALACE OF CASERTA (ITALY)

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Fairy rings, a common occurrence in grasslands, manifest as circular patterns resulting from the subterranean expansion of fungal mycelium. Their presence is discernible through alterations in vegetation or the emergence of sporophores. The saprobic activity of mycelia, which forms fungal fronts, induces modifications in soil biotic and abiotic characteristics, thereby influencing species coexistence and functioning as an ecosystem engineer. Fairy rings offer an opportunity to trace the dominant fungal mycelium in soil, rendering them an optimal experimental system for investigating the behavior of fungal saprotrophs in the soil ecosystem. In the Royal Palace of Caserta's gardens, we scrutinized variations in soil biotic and abiotic properties linked to the mycelia of three fairy ring fungi. Five fairy rings were sampled at three distinct positions within the ring: an area devoid of fungal mycelium (OUT), at the fungal front (FF), and post-fungal front passage (IN). Comprehensive analyses, including metagenomic assessments and soil chemical and biochemical surveys, were conducted. Metagenomic analysis unveiled a significant simplification in the soil mycobiome and a consistent alteration in the prokaryotic community. The former is simplified due to the competitive strategies of the dominant mycelium, while the latter is structured based on the fairy ring-forming species, the fungal decomposition capacity, and the legacy effect of fungal front widening. Concurrently, acidification and an increase in bioavailable nutrients were noted. Microbial biomass and soil respiration exhibited heightened activity in the presence of fungal mycelium. The enzymatic activities of laccase disclosed distinctive lignin degradation strategies. The findings suggest that the mycelium of grassland saprotrophs employs a resource monopolizing strategy, leading to significant alterations in the biotic and abiotic characteristics and species richness of grassland soil. This research enhances our understanding of the pivotal role of this taxon in soil system dynamics and informs conservation policies for grasslands.

KEYWORDS: fungal fronts; basidiomycota; next generation sequencing; *Marasmius oreades; Amanita vittadini; Clitocybe collina; f*ungal saprotrophs

GR95

INACTIVATION OF DIPHTHERIA TOXIN BY SITE-DIRECTED MUTAGENESIS

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Diphtheria toxin is a single polypeptide chain produced by toxigenic strains of *Corynebacterium diphtheriae* that causes the disease diphtheria in humans by gaining entry into the cytoplasm of cells and inhibiting protein synthesis. Formaldehyde (chemical) detoxification converts diphtheria toxin into toxoid, which is used in diphtheria vaccine production. Recombinant, genetically detoxified diphtheria toxin is superior in terms of safety and purity, but it has still not found its application in recombinant diphtheria vaccine production. Both chemically and genetically inactivated forms of the diphtheria toxin have proven effective as protein carriers in conjugate vaccines. The goal of this study was to create a plasmid construct which can be used to express a genetically inactivated diphtheria toxin. Gene coding for diphtheria toxin was cloned into pMALHisEk expression vector and introduced into DH5α competent *Escherichia coli* cells. Three site-directed point mutations, which led to three amino acid substitutions (G52E-substitutes glycine with glutamic acid, G79D- substitutes glycine with aspartic acid, E148D- substitutes glutamic acid with aspartic acid) were conducted. A single G52E amino acid substitution is responsible for the loss of the enzymatic activity of the diphtheria toxin. G79D is recognized as a good candidate site for combining with other mutations in vaccine development and E148D may be a good candidate as carrier protein because it could reduce both the stability of NAD binding and catalytic activity of the enzyme. Each individual mutation is sufficient for toxin inactivation, but together they ensure non-toxicity, preventing reversion to the wild-type sequence. All mutations were confirmed by DNA sequencing. Recombinant diphtheria toxoid could serve as a potential vaccine epitope or protein carrier for conjugate vaccines. Further optimization of recombinant protein expression in *Escherichia coli* should provide sufficient quantities of soluble recombinant protein for further testing of its safety, immunogenicity and protection.

KEYWORDS: diphtheria toxin; genetic detoxification; site-directed mutagenesis; vaccine

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PP96

SHORT-CHAIN FATTY ACID-PRODUCING *FAECALIMONAS SP.* NGB245 STRAIN REGULATES THE EXPRESSION OF NEURONAL ACTIVITY-REGULATED GENES AND ATTENUATES THE SYMPTOMS OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Alterations in gut microbiota and deregulation of the gut immune system are recognized as important events in autoimmune diseases. The knowledge about the important role of anaerobic gut bacteria that produce short-chain fatty acids (SCFAs), in the regulation of intestinal barrier and immune response made a way for the development of microbiota-based interventions. Our research aimed to isolate the strains with the potential to produce SCFAs, from healthy volunteer fecal material, and to test their effects on IL-8 production in the culture of intestinal epithelial cells (Caco2) as an *in vitro* system imitating initial intestinal inflammation, the effects on the expression of neuronal activity-regulated genes of *Caenorhabditis elegans*, and the effect on the development of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. Three isolated butyric acid (BA)-producing strains, and three acetic acid (AA)-producing strains diminished the production of IL-8 in Caco-2 cells treated with IL-1β/TNF-α. Further, all BA-producing strains stimulated the expression of important neuro-related genes in *C. elegans*. Based on the strongest effects in these assays an isolate identified as *Faecalimonas* sp. NGB245 strain was further tested in EAE model. The oral treatment of EAE-induced mice with this strain for 16h per day for 15 days resulted in alleviated daily clinical scores, maximal clinical scores, and the duration of the illness in comparison to the effect of media used for strain cultivation. These results point to the potential of NGB245 to modify the gut-brain axis opening the field for future development of microbiota-based therapy for the diseases associated with immune response dysfunctions.

KEYWORDS: microbiota-based therapy; short-chain fatty acid (SCFA); intestinal barrier; immune response; autoimmune disease

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PP97

SIGNIFICANCE OF CYTOMEGALOVIRUS GB GENOTYPES IN ADULT PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION: INSIGHTS FROM A SINGLE-CENTRE INVESTIGATION

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Cytomegalovirus (CMV) infection is a major clinical issue after allogeneic hematopoietic stem cell transplantation (HSCT). The CMV envelope glycoproteins are key in viral pathogenesis; the glycoprotein B (gB) encoded by the UL55 gene might be an important determinant of viral virulence and disease severity marker in patients treated with allogeneic HSCT. Our aim was to investigate the molecular diversity of CMV gB and inquire into the associations between UL55 gene variations and clinical manifestations in adult patients treated with allogeneic HSCT. The study included fifty-nine adult patients treated with allogeneic HSCT. Peripheral venous blood was sampled typically per week, with detection of CMV performed by quantitative Real-Time PCR. Multiplex nested PCR was used to determine specific gB genotypes, which were then statistically compared vis-à-vis specific clinical variables. The most prevalent genotypes were gB1 and gB4 (11/40, 27.5%). Patients with genotype gB1 infection had earlier platelet engraftment ($p<0.033$) and less frequent Minimal/measurable residual disease post HSCT than those without this genotype. Patients with gB4 glycoprotein infection had a significantly lower CD4+/CD8+ ratio at D90 ($p<0.026$). Interestingly, patients with gB5 glycoprotein infection had shorter overall survival from base condition diagnosis ($p<0.042$), as well as shorter overall survival after HSCT ($p<0.036$). Neither acute nor chronic GvHD were noted more frequently in those with mixed genotype infection ($p>0.05$). Our study points to variations in the viral UL55 locus imparting both beneficial (earlier platelet engraftment, less frequent MRD post-HSCT) and adverse effects (shorter overall survival, more frequent acute GvHD, less frequent 100% chimerism at day 90) to the transplanted host. Comprehensive molecular investigations are necessary to validate this apparent duality, as the potential benefits of CMV could perhaps be utilized for the benefit of the patient in the future.

KEYWORDS: cytomegalovirus; gB genotype; hematopoietic stem cell transplant; adult; GvHD

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PP98

THE SIGNIFICANCE OF MOLECULAR DIAGNOSTIC IN ADVANCED HIV INFECTION – CLINICAL CASE

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Opportunistic infections are still frequent and severe among patients with advanced HIV infection. Accordingly, it is essential to perform rapid microbiological diagnostic tools aiming to introduce specific antimicrobial and antiretroviral therapy as soon as possible. We performed a retrospective case study of a patient presented with advanced HIV infection in which the rapid PCR diagnostic methods were performed in order to diagnose different patogens. The HIV infection diagnosis was confirmed with quantitative PCR HIV RNK, VL (viral load) =34300 cp/mL, along with the CD4 cells count 5/µL. In adition CMV, as an opportunistic infection was detected, according to the VL in different samples: PCR CMV DNA = 21200 IU/mL (blood), PCR CMV DNA = 2295IU/ml (CSF) and PCR CMV DNA = 178 IU/ml (BAL). Also, CMV was detected in aqueous humor sample with BioFire Multiplex Meningitis/Encephalitis PCR panel which distinguish bacterial from viral infection. With quantitative real-time PCR Pneumocystis jirovecii DNK, in the patient's sputum Pneumocystis jirovecii infection was proven (1286967 cp/mL). Meanwhile, patient was Norovirus positive on BioFire Multiplex PCR Gastrointestinal panel. In several nasopharyngeal swabs in couple of months, SARS-CoV-2 virus was detected with SARS-CoV-2 RT-PCR. For AIDS patients with suspected opportunistic infections, PCR testing can provide early, noninvasive, and rapid microbiological diagnosis. The results of these PCR test were available to clinicians within few hours after sampling, including resistance profile for certain microorganisms. These rapid molecular diagnostic methods may lead to a more precise antimicrobial treatment.

KEYWORDS: HIV; molecular diagnostic methods; multiplex PCR

ALTERNATIVE APPROACHES IN ANTIMICROBIAL CONTROL

IL99

BIOMARKER GUIDED ANTIFUNGAL THERAPY: A CURRENT STATE OF LABORATORY MYCOLOGY AND ANTIFUNGAL MANAGEMENT

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Invasive fungal diseases are defined as systemic infections that occur as a result of yeast or mold invasion into deep tissues. In contrast to superficial fungal infections, they are significantly less common with an estimated incidence rate of 6 per 100,000 persons per year. Invasive fungal diseases are associated with a high mortality rate, especially in the high-risk patient population. Early diagnosis of these diseases is crucial because any delay can be fatal. Conventional mycological methods based on isolation are often time-consuming and require invasive diagnostic procedures to obtain an optimal sample. To overcome this problem, several diagnostic approaches have been developed to facilitate the early diagnosis of the most common invasive fungal diseases. These less invasive diagnostic methods are based on the detection of serological and molecular biomarkers. Currently, there are only a few specific biomarkers for the most common pathogens such as mannan for *Candida* spp., galactomannan for *Aspergillus* spp., and glucuroxylomannan for *Cryptococcus* spp. In addition, there is a panfungal biomarker 1,3-*β*-D-glucan, and also molecular biomarkers have recently been included in the criteria for the classification of invasive fungal diseases. Today, these biomarkers are increasingly used in the diagnosis of invasive fungal diseases in high-risk patients with the aim of early disease detection and timely initiation of specific antimycotic therapy. There is more and more evidence that a biomarker guided approach to the treatment of invasive fungal diseases enables the safe and timely application of adequate antimycotic therapy. Emergence of resistance to antifungal drugs in *Candida* spp. and *Aspergillus* spp. as the most common cause of invasive fungal diseases is worrisome, and requires increased use of antifungal susceptibility testing in order to manage antifungal drugs and make correct clinical decisions. This approach enables a personalized strategy in the treatment with achievement of optimal outcomes in patients.

KEYWORDS: invasive fungal diseases; biomarkers; antifungal therapy; antifungal drug resistance

IL100

BACTERIOPHAGES OF MULTIDRUG-RESISTANT NOSOCOMIAL PATHOGENS – BELGRADE EXPERIENCE

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Antimicrobial resistance (AMR) arises when bacteria and other microbes stop responding to medications. AMR is now recognized as one of serious global health threats, repeatedly appearing in the World Health Organization's (WHO) lists of urgent global health challenges, including the 2024 list. It is taking a fatal toll – nearly 5 million deaths globally per year are associated with AMR, encompassing 1.27 million directly attributed to AMR. The COVID-19 pandemic paved the way for aggravation of bacterial AMR – primarily due to enhancement in unspecific and unjustified prescription and use of broad-spectrum antibiotics, resulting in what is now recognized as „silent pandemic of AMR". Bacteriophages (phages) are natural and specific predators of bacteria - viruses that can infect, replicate inside and lyse arguably any bacteria. Their therapeutic potential is being hastily evaluated through different approaches: in silico, in vitro, ex vivo and in vivo – in laboratory animals as well as in human case and clinical studies. Although the results are promising, bacteria rapidly develop resistance against phages, which why the isolation and research of new phages is needed. Our work is concentrated on three bacterial species for which critical priority by WHO has been declared – carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Twenty distinct pathogenic strains of *A. baumannii*, 6 *K. pneumoniae* and 6 *P. aeruginosa* were used as targets for bacteriophage isolation, and total of 14, 22 and 8 potentially distinct phages were collected, respectively. All strains were nosocomial isolates obtained from various tissues, including from terminally ill patients. Six phages were characterized in detail. In particular, phage vB_AbaM_ISTD was applied against *A. baumannii* in zebrafish embryo model of systemic infection, and demonstrated powerful therapeutic potential, eradicating the infection. Interestingly, its DNA was characterized with highly modified thymidine (amassing 1228 Da), making it the largest non-canonical deoxynucleoside reported so far.

KEYWORDS: antimicrobial resistance (AMR); nosocomial infections; bacteriophages, zebrafish model

IL101

NOVEL BACTERIOPHAGE-BASED DEPOLYMERASE STRATEGIES TO CONTROL *A. BAUMANNII*

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Carbapenem-resistant *Acinetobacter baumannii* are bacteria resistant to nearly all antibiotics and difficult to remove from the environment. This bacterium displays diverse virulence factors (e.g. capsules) to protect themselves from environmental pressures, including viral predation. As antibiotic resistance represents a great challenge for health care worldwide, novel strategies to control infection by drug-resistant bacteria are urgently needed. Bacteriophages (viruses of microbes), due to their bacterial predatory nature, represent a natural toolbox on promising antibacterial proteins. Depolymerases are bacteriophage-derived proteins with high antibacterial potential and the focus of this talk. Depolymerases are proteins located in the bacteriophage tails used to recognize and degrade capsules of their hosts, to facilitate infection. These recombinant enzymes have been found to be highly specific, stable and refractory to resistance, as they do not kill bacteria per se, instead they remove bacterial capsules, turning them into avirulent form easily managed by the immune system. Thus, depolymerases represent an innovative anti-virulence approach to control *A. baumannii* infections. Moreover, as these determine the host range, bacteriophage genomes can also be engineered to alter host specificities. Using homologous recombination, depolymerase-encoding genes can be swapped to tune the host range of the bacteriophages targeting *A. baumannii* serotypes. To advance research in the field, bioinformatic tools can also assist the identification of novel bacteriophage depolymerases (e.g. PhageDPO) and to predict phage-host pairs to choose effectives phages for therapy. Such information is being compiled in a collaborative tool called PhageKDB open portal to phage community.

KEYWORDS: antibiotic-resistance; bacteriophage; antibacterial proteins; bioinformatic tools

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IL102

DIAGNOSIS OF INTESTINAL HELMINTH INFECTIONS: STRENGTHS AND LIMITATIONS

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More than a quarter of the world's population is at risk of intestinal helminth infections (IHIs) caused by *Ascaris lumbricoides*, *Enterobius vermicularis*, hookworms, *Trichuris trichiura* and *Strongyloides stercoralis*. Infected children and adults present with a range of medical and surgical conditions, particularly in immunocompromised patients. The intestinal parasites have similarities in their mode of transmission and life cycle. Diagnosis of IHIs requires knowledge of the parasites' geographical distributions, and an understanding of the varied, and often overlapping clinical picture of disease. The stool test is the primary way of diagnosing IHIs. After collecting stool sample, usually formalin-ethyl acetate sedimentation concentration technique is performed, nowadays with commercial systems. After formalin - ethyl acetate sedimentation concentration technique, direct smear examination should be performing with and without Lugol's liquid, examined under a microscope at 400 x magnification. Parasitological-based laboratories commonly use egg concentration techniques such as Kato-Katz and FLOTAC technique. According to morphological features of different protozoa and helminth eggs, diagnosis is made by experienced laboratory staff. Exception of this kind of stool examination is the diagnosis of entrobiasis which should be done by Scotch tape test, where collecting pin-worm eggs from perianal skin area with transparent sticky tape is performed, followed by microscopy. The current guidelines for IHIs diagnosis, suggest multiple, at least three consecutive stool samples to be checked in order to cumulative higher probability of finding specific parasitic ova. The practice of examination of only one stool sample very often leads to misdiagnosis. Co-infections with multiple parasites are common in endemic areas, making diagnosis challenging. After accurate diagnosis, there are contemporary guidelines for treatment of IHIs, sometimes challenging, especially in pediatrics population.

KEYWORDS: helminths infection; ascariasis; enterobiasis; trichuriasis; strongiloidosis

IL103

BIOFILMS IN PREMISE PLUMBING SYSTEMS - CURRENT CHALLENGES AND POTENTIAL SOLUTIONS

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The function of a drinking water distribution system is to deliver potable water to all customers in sufficient quantity and with acceptable quality. Biofilms collected from premise plumbing systems have been shown to contain diverse groups of microorganisms belonging togroup of opportunistic premise plumbing pathogens (OPPPs). In adition that they can cause disease, bacterial biofilms within plumbing are associated with the deterioration of physico-chemical water quality due to degradation and corrosion of pipe materials. As a result, premise plumbing conditions can amplify the potential public health risk relative to the drinking water distribution system. Pathogenic bacteria previously recognized as biofilm-associated OPPPs include *Legionella pneumophila*, nontuberculous mycobacterial (NTM) species, and *Pseudomonas aeruginosa*. Current approaches to water disinfection, including chlorination, use of chloramine, chlorine dioxide and ozone, thermal method, copper-silver ionization, and ultraviolet (UV) radiation are not effective in removing biofilm from water systems. A new way of application of standard methods (their combination) or new innovative methods are necessary in the fight against these opportunistic pathogens. The potential application of combinations of standard methods such as chlorination and UV radiation or ozonation will be presented, as well as the application of photodynamic inactivation against the selected opportunistic pathogens.

KEYWORDS: water biofilms; *Legionella*; NTM; *Pseudomonas*; water disinfection

IL104

OVERVIEW OF THE CURRENT SITUATION ON EPIDEMIOLOGY AND MANAGEMENT OF IFI AND FUTURE PERSPECTIVES

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Invasive fungal infections (IFI) present significant challenges in healthcare, with increasing incidence rates globally. This abstract provides a comprehensive overview of the current epidemiological landscape of IFIs, as well as strategies for their management, and offers insights into future directions for research and treatment modalities. Diagnosis of IFIs is crucial for effective management. It typically involves clinical assessment, laboratory tests, and imaging studies. Microbiological confirmation is standard but can be time-consuming. Rapid diagnostic assays and advanced imaging modalities play a vital role in early detection and treatment. Integration of these tools into clinical practice is essential to improve patient outcomes. Effective management of IFIs necessitates a multidisciplinary approach, incorporating antifungal therapy, infection control measures, and supportive care strategies. Antifungal agents play a central role in treatment, with recent advancements in drug development enhancing therapeutic options. However, the emergence of antifungal resistance poses a significant threat, underscoring the importance of judicious antimicrobial use and surveillance programs. Looking ahead, future perspectives in the field of IFIs encompass a range of innovative approaches, including the development of novel antifungal agents, advancements in diagnostic techniques, and the exploration of immunotherapeutic strategies. Additionally, the integration of precision medicine approaches tailored to individual patient characteristics holds promise for optimizing treatment outcomes and reducing the impact of IFIs on public health. In conclusion, this abstract highlights the evolving landscape of IFIs, emphasizing the importance of continued research efforts and collaborative initiatives to address the challenges posed by these complex infections and improve patient outcomes globally.

KEYWORDS: antifungal resistance; multidisciplinary approach; infection prevention; precision medicine; immunotherapeutic strategies

IL105

NEW APPROACHES IN THE TREATMENT OF CHRONIC BACTERIAL INFECTIONS

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The rapid emergence and spread of multi-drug-resistant pathogens present a global healthcare challenge. One common cause of resistance and/or tolerance to antibiotics is biofilms, a complex communities of bacteria embedded in a self-produced matrix. Biofilm formation and maturation are regulated by quorum sensing, a cell density-dependent communication system that relies on the synthesis, diffusion, and detection of small signaling molecules - autoinducers (AIs). Quorum quenching (QQ) enzymes that cut AIs emerged as a promising strategy for persistent bacterial infections. However, a significant drawback for the use of QQ enzymes as therapeutics is their poor stability and efficacy in vivo. Since one of the major health issues linked to biofilm development is persistent wound infections, our goal was to improve enzyme properties by immobilizing it on a natural biopolymer to make it suitable for use as a wound dressing. The best candidate for immobilization was YtnP lactonase from *Bacillus paralicheniformis* ZP1, as in concentrations higher than 25 µg/mL it improved the survival of *Pseudomonas aeruginosa* PAO1-infected zebrafish, rescuing 80% of embryos. When combined with tobramycin or gentamicin, the survival rate of zebrafish embryos increased to 100%. Purified YtnP lactonase at a concentration of 1 mg was immobilized on 10 mg of polymer disks by crosslinking with glutaraldehyde. Specific modifications of the polymer were also made to eliminate the use of glutaraldehyde, which is a skin irritant. In in vivo experiments on a murine chronic wound model, immobilized enzyme inhibited biofilm development, cleared already formed biofilms, and overall improved wound healing. These results provide a foundation for the development of advanced wound dressings that will prevent infection development in wounds and enable proper therapy for infected chronic wounds.

KEYWORDS: biofilm; chronic wounds; *Pseudomonas aeruginosa* PAO1; YtnP lactonase; immobilization

IL106

PHAGE-HOST INTERACTION WITH CELLS IN DIFFERENT METABOLIC STATES: A S. *EPIDERMIDIS* CASE

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In nature, bacteria are not frequently found in the exponential state of growth. One particular issue is that the efficacy of antimicrobials, including phages, is always tested against bacterial cells at their highest growth rate. The majority of bacterial biomass occur in the form of a biofilm. Biofilms have a high tolerance to antimicrobial agents, mainly, due to the low metabolic activity of the biofilm cells and the presence of the biofilm matrix. To date, only a few staphylococcal phages were shown to be efficient against biofilms. In addition, there are only two reports of phages capable of successfully infecting cells in a low metabolic state. In this study, the *Staphylococcus epidermidis* phage SEP1 was used as a model to study phage-bacteria interactions. We demonstrated that besides some interesting features, this phage showed a reduced activity against biofilms. We clearly showed that the biofilm matrix was the main factor influencing SEP1 inefficacy against biofilms. In addition, SEP1 was shown to be highly effective against persister cells, biofilm-released cells and stationary-phase cells. This rare phenomenon was very recently studied through an RNA-seq analysis, where we demonstrate that SEP1 successfully hijacks the transcription machinery of its host, activating important metabolic and biosynthetic processes in stationary cells necessary for its effective replication. The gathered data provides important insights for a better implementation of phage therapy, since phages with ability to infect stationary cells could be more efficient in the treatment of patients with biofilm-related chronic infections.

KEYWORDS: bacteriophages; biofilms; stationary-phase cells; phage/bacteria interactions; RNA-seq

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IL107

UNVEILING THE JANUS-FACE OF BACTERIOPHAGES: A DUAL PERSPECTIVE ON ANTIBACTERIAL THERAPY

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The rise of antibiotic-resistant bacteria has propelled bacteriophages into the spotlight as potential therapeutic agents. Bacteriophages, viruses that infect bacteria, exhibit a Janus-like duality in antibacterial therapy. On one hand, they serve as vehicles for horizontal gene transfer, contributing to the spread of antibiotic resistance, virulence factors and toxins. On the other hand, they offer a promising avenue for combating antibiotic-resistant infections through their innate antimicrobial properties. Bacteriophages, through transduction, can mediate the transfer of antibiotic resistance genes among bacterial populations. This phenomenon underscores the intricate relationship between bacteriophages and antibiotic resistance, presenting a formidable challenge in the clinical landscape. Understanding the mechanisms and dynamics of phage-mediated gene transfer is crucial for mitigating the risk of exacerbating antibiotic resistance while harnessing the therapeutic potential of bacteriophages. Despite their role in genetic transfer, bacteriophages possess inherent antimicrobial activity that can be leveraged for therapeutic purposes. Their specificity in targeting bacterial hosts offers a tailored approach to infection treatment, minimizing collateral damage to the host microbiota. Moreover, bacteriophages have the capacity to evolve alongside bacteria, potentially overcoming resistance mechanisms and maintaining efficacy over time. This presentation delves into the Janus-faced nature of bacteriophages in antibacterial therapy, emphasizing the dual roles they play in the context of antibiotic resistance. Through an exploration of phage-mediated gene transfer and their antimicrobial properties, we aim to elucidate the complexities and opportunities associated with bacteriophage therapy. By dissecting the Janus-face of bacteriophages, we can develop strategies to maximize their therapeutic benefits while mitigating the risks posed by antibiotic resistance dissemination. This comprehensive understanding will pave the way for the effective integration of bacteriophages into the antimicrobial armamentarium, offering new hope in the fight against antibiotic-resistant infections.

KEYWORDS: bacteriophages; phage therapy; antibiotic resistance

IL108

MICROFUNGI AS A TARGET AND SOURCE OF VALUABLE COMPOUNDS

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Members of the extensive Kingdom of Fungi are characterized with wide possibilities in their biosynthetic pathways. This results in production of compounds with unique chemical features and even more distinctive biological properties. Various primary and secondary metabolites, including sugars and their derivatives, quaternary amine bases, isoprenoids, sterols, phenolic acids, ergot alkaloids and others have been praised for their possible application in various aspects of pharmaceutical industry. Ever since the accidental discovery of penicillin at the beginning of the 20th century, microfungi have been increasingly utilized as to evaluate their potential as producers of compounds with preferable antibacterial action. Along with the known fact that various *Penicillium* species may inhibit growth of numerous pathogenic microorganisms, as we earlier demonstrated inhibitory effect of certain species on the growth of eleven clinical isolates of *Helicobacter pylori*. However, this research has to be reinforced as to apply the obtained results into the clinical practice. Along with this, the possibility of extraction of bioactive organic acids, sugars, proteins, glucan, phenolic acids, and tocopherols from different culturing media is also important for obtaining of new bioactive compounds. The results showed that distinctions in chemical composition of culturing media may alter biochemical responses in microfungi, leading to differences in yield and presence of certain chemical compounds. This further implies that by using certain media for cultivation that correspond to certain nutritional requirements of microfungi, we can guide them to produce substances with bioactive properties we are interested in. Nonetheless, more has to be explored in order to have benefit in pharmaceutical industry from these results. Research showed that microfungi may be promising sources of bioactive compounds with application in antimicrobial drugs discovery, regardless to the fact that some of them have been cosidered hazardous for human health due to production of certain mycotoxins. Eventhough we live in an era of biotechnological revolution, Paracelsus basic principle of toxicology: "The dose makes the poison" has been validated once again. Today, thanks to the achievements in the field of metabolomics and other technologies, elucidation of chemistry and underlying mechanisms of action may very well be essential in creating novel treatment approaches in the future. This has to correspond with advances in fungal fermentation technologies, separation techniques, and structural determination as to identify novel bioactive secondary metabolites derived from microfungi.

KEYWORDS: microfungi; bioactive compounds; bioactive properties

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IL109

LEGIONELLA PNEUMOPHILA - JOURNEY FROM THE ENVIRONMENT TO HUMAN MACROPHAGES

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Legionella pneumophila is an aquatic Gram-negative bacterium that multiplies in amoebae as its main natural host. When humans inhale water aerosols contaminated with *L. pneumophila*, the bacteria multiply in the alveolar macrophages and cause pneumonia, known as Legionnaires' disease. The intracellular lifestyle of *L. pneumophila* in amoebae and macrophages is very similar. The bacterium multiplies within the cell in a compartment called the Legionella-containing vacuole (LCV) and is protected from degradation by external stimuli. Contamination of water supply systems with *Legionella* spp. is a serious health problem. This review will summarize the frequency of colonization by *L. pneumophila* in various water sources (swimming pools, wellness centres, hotels, apartments, campsites, rehabilitation centres, ferries and cooling towers) in Primorje-Gorski Kotar County, Croatia, and the unique lifestyle of Legionella within the cell and environment.

KEYWORDS: *Legionella*; amoebae; macrophages

IL110

HERBAL PRODUCTS AS AN ALTERNATIVE TO ANTIBIOTICS: APPLICATION POSSIBILITIES AND LIMITATIONS

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Antimicrobial resistance (AMR) has developed as one of the top 10 global public health threats facing humanity. As the nosocomial bacterial strains are being increasingly resistant to most clinically available antibiotics, there is a constant need for exploration of new substances that could kill them or inhibit their growth, or alternatively inhibit some of their essential virulence factors to counteract the lack of new antibacterials and the rise of antibiotic resistance, plants could represent a potential solution. Plants produce a variety of bioactive secondary metabolites that could be used to fuel the future discovery pipeline. Aim of the present study was to examine inhibitory activity of the supercritical extract of *J. communis* L. green pseudofructus (7SCO2) against the growth, biofilm production and several virulence factors of significant nosocomial bacterial pathogens. The extract was obtained by fractional extraction with supercritical CO2, and the qualitative and quantitative analysis was performed using the GC-FID/MS method. Clinical isolates of *Pseudomonas aeruginosa, Acinetobacter baumannii*, *Staphylococcus aureus* (methicillin-sensitive-MSSA and methicillin-resistant - MRSA), *Enterococcus faecalis*, and *Klebsiella pneumoniae*, as well as their antibiotic resistance profiles, were obtained from the Clinical Hospital Centre "Dr Dragiša Mišović Dedinje". Minimum inhibitory concentrations (MICs) of the 7SCO2 were determined by broth-microdilution method. Examination of the anti-adhesive effect of the extract was carried out using the spectrophotometric method. The pyocyanin production of *Pseudomonas aeruginosa* was determined by the method described by Rampioni et al. Most significant findings of this study are potent antivirulence activity of the 7SCO2 against *P. aeruginosa* through the inhibition of pyocyanin production. In addition, the biofilm production of *A. baumannii* was inhibited by the 7SCO2 in concentration 50 µg/mL. Finally, notable antivirulence activity of the 7SCO2 against *E. faecalis* and *S. aureus* was detected, since it significantly inhibited collagen and laminin adhesion of these pathogens.

KEYWORDS: *Juniperus communis;* biofilm; adhesion; pyocyanin

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IL111

BIOCONTROL ACTIVITY OF PLANT PRODUCTS AGAINST PLANT PATHOGENS

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Phytopathogenic fungi are one of the leading infectious agents in plants causing alterations during the different plant stages growth, post-harvest and even during storage. Application of chemical fungicides is the most prevalent in crop protection but such measures are not appropriate for treatment of medicinal plants destined for herbal products, because they are prohibited according to the current legislation in our country. This have encouraged intensive research in the field of natural fungicides development, especially plant based, as an ecologically safer approach to plant pathogen control due to the limited risk to the environment and humans, but there is still not enough scientific knowledge about their practical application. From that starting point, a great number of essential oils and plant extracts were screened in our investigation in order to develop effective plant-based fungicides against the fungal pathogens from genera *Aspergillus*, *Fusarium* and *Alternaria* that are most often isolated from medicinal plants but also from their seeds. Except extracts from medicinal plants we also investigated extracts from industrial plants that after harvest have waste with potentially valuable ingredients, such as kale (remains of leaves), horseradish (leaves and root), pods and grains of faba bean, thistle leaf. Results showed that beside thyme, origano and savory essential oils, high fungicidal activity exhibited also rosemary, myrtle, cloves, laurel, cumin, peppermint and cinnamon essential oils reducing both, spore germination and mycelial growth at very low concentration. Among the extracts, extracts of leaves and roots of horseradish, savory tea, nettles and horsetail showed the highest inhibitory activity, slightly less extracts of kale, thistle leaves and bean pods. The tested moulds did not show the same sensitivity to oils and extracts, which indicates the need for a mixture formulation in order to achieve the best possible effect. By examining the fumigant activity of selected essential oils and extracts, as well as their combinations, it was shown that the level of fungal contamination of infected plant drugs was reduced above 80%, which indicates their huge potential as biofumigant. Current research goes in several directions: a) encapsulation of various combinations of essential oils and extracts to enhance their stability, efficasy and controlled release b) examination the possibility of using encapsulated mixtures of essential oils and extracts in the prevention of contamination of herbal drugs with pathogenic fungi during long storage and influence on their organoleptic properties d) greenhouse and field tests in order to prevent pre-harvest contamination and deterioration.

KEYWORDS: phytopathogenic fungi; biocontrol activity; essential oils; plant extracts; biofungicides; biofumigants

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HOST-MICROBIOTA INTERPLAY REGULATES EPITHELIAL BARRIER FUNCTION AND WOUND HEALING

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Skin microbiome emerged as an important factor which can balance tissue repair process and wound healing. Recent evidence suggest that intracellular bacterial localization could be associated with the aberrant healing observed in patients with chronic wounds, while therapeutics targeting intracellular bacteria remain limited. Probiotic lactobacilli and their bioactive lysates (postbiotics) are well known for their role in maintenance of gut epithelial homeostasis. Hence, in this study we focused to understand the mechanisms of cutaneous response to fourteen postbiotics derived from different lactobacilli to reduce intracellular *Staphylococcus aureus* colonization and promote healing. *Latilactobacillus curvatus* BGMK2-41 demonstrated the most efficient capability to reduce intracellular infection by *S. aureus* in keratinocytes *in vitro* and infection of human skin explants. Reduction of bacterial number was followed by upregulation of the expression of antimicrobial response genes. Furthermore, BGMK2-41 postbiotic treatment stimulates keratinocyte migration *in vitro* and increases expression of anti-inflammatory cytokine IL-10, promotes wound closure and strengthens the epidermal barrier via upregulation of tight junction proteins in a human *ex vivo* wound model. Altogether, this study provided evidence that postbiotics could stimulate fortification of epithelial barrier to suppress dissemination of intracellular pathogens which can be used as a novel approach to treat dermatologic and wound healing disorders associated with persistent infections.

KEYWORDS: lactobacilli; postbiotics; *Staphylococcus aureus;* wound healing; skin barrier

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NON-TAILED ICOSAHEDRAL PHAGES AS ANTIBACTERIAL AGENTS

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Non-tailed icosahedral phages from the families *Microviridae* (Escherichia phage phiX174), *Tectiviridae* (Enterobacteria phage PRD1) and *Fiersviridae* (Escherichia phage MS2 and Escherichia phage Qbeta) have not yet been thoroughly examined as possible antibacterial agents. The objective of the study was to assess antibacterial potential of these phages. Antimicrobial and antibiofilm effect of four phages was investigated in addition to determination of lytic spectra, the frequency of bacterial insensitive mutant appearance and analysis of phage safety. Out of the four phages tested against their respective hosts, only phiX174 showed high rate of bacterial growth inhibition by more than 90% even at very low multiplicity of infection and only this phage significantly prevented biofilm formation. Despite the fact that presence of potentially deleterious genes was not determined in analysed phages, their lytic spectrum is exceedingly narrow. Since phiX174 showed better antibacterial characteristics compared to the other tested phages, it was investigated in combinations with antibiotics. Synergistic effect was detected with cephalosporins (ceftriaxone and ceftazidime), fluoroquinolones (ciprofloxacin), macrolides (azithromycin) and chloramphenicol, while lack of synergism was observed with aminoglycosides (gentamicin), tetracycline, and amoxicillin-clavulanic acid. The results indicated that administration of agents concurrently yields greater results than consecutive treatment with one agent applied first. Investigation of the incidence of phiX174 bacteriophage insensitive mutants revealed a 10^{-3} frequency of mutations. Since analysed non-tailed phages have very narrow lytic spectrum and high rate of resistant mutants occurrence, their antibacterial potential is limited. However, antibacterial potential of *Microviridae* in combination with antibiotics may be considered in the future.

KEYWORDS: icosahedral phages; phiX174; biofilm; synergy; lytic spectrum

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GR114

BIOCOMPATIBILITY OF *ACHILLEA MILLEFOLIUM* METHANOLIC EXTRACT AND ITS ANTIMICROBIAL ACTIVITY

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The problem of rapidly growing antimicrobial resistance worldwide compromises the effectiveness of conventional antibiotics and leads to the constant search for new antimicrobial agents from natural sources, especially plants. *Achillea millefolium* L. (Asteraceae), traditionally known as yarrow, is an herbaceous perennial plant predominantly distributed in the northern hemisphere and widely used in traditional medicine in various countries. Due to the well-known benefits of this plant in folk medicine, the aim of this study was to investigate chemical composition of yarrow methanolic extract, its antimicrobial activity and biocompatibility, all in order to provide justification of its traditional use. Chemical analysis acquired by quantitative LC–MS analysis revealed 28 phenolic compounds in the extract, predominantly caffeoylquinic acids and flavonoids. The results of microdilution assay showed moderate antimicrobial activity with highest MIC values for *L. monocytogenes* (1.25 mg mL^{-1}) and *C. albicans* (2.5 mg mL^{-1}). Further, investigation of the antibiofilm effects, carried out using crystal violet staining, showed that methanolic extract significantly inhibited biofilm formation of *L. monocytogenes* with the highest inhibition of 73 %, but did not disrupt pre-formed biofilm. On the contrary, inhibition of *C. albicans* biofilm formation was not detected. Additionally, the extract induced the reduction in *L. monocytogenes* motility up to 58 %. In order to examine whether yarrow extract is safe for use, cytotoxic potential was investigated by MTT assay on normal fetal fibroblasts (MRC-5). As it is crucial that the potential antimicrobial agent possesses selective toxicity, antimicrobial selectivity index (SIM) was calculated and obtained results revealed that the extract exhibited greater toxicity towards *L. monocytogenes* and *C. albicans* than to human cells. Based on the obtained results, the *A. millefolium* extract could be considered as a good candidate for a novel antimicrobial agent, but further research into its underlying mechanisms is necessary.

KEYWORDS: *Achillea millefolium*; antimicrobial; antibiofilm; listerial motility, cytotoxicity

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GR115

ANTIMICROBIAL POTENTIAL OF SPOTTED KNAPWEED (*CENTAUREA STOEBE* L., ASTERACEAE) FROM SERBIA

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Spotted knapweed (*Centaurea stoebe* L., Asteraceae) is a biennial/perennial herbaceous plant. The species is widespread in Serbia – it grows near roads and railroad lines, on rocky and sandy places, on arable land, as well as in grassy places. Spotted knapweed is native to Eastern Europe but has been introduced to the New World where the species is considered invasive. The aim of this study was to evaluate the antimicrobial potential of different extracts of *C. stoebe*. The plant material was collected during the flowering period in July 2021 near Kruševac, Serbia. The antimicrobial activity of diethyl ether (Et2O), methanol (MeOH), 70% ethanol (EtOH), ethyl-acetate (EtOAc), 50% acetone (Me2CO) and dichloromethane: methanol (DCM: MeOH, 1:1) extracts of aerial parts of *C. stoebe* are evaluated on four bacterial and four *Candida* strains using microdilution method. Bacterial strains were: *Escherichia coli* (ATCC 35210), *Klebsiella pneumoniae* (ATCC 13883), *Staphylococcus aureus* (ATCC 11632), and *Pseudomonas aeruginosa* (PAO1), while *Candida* strains included: *Candida albicans* (ATCC 10231), *C. parapsilosis* (ATCC 22019), *C. tropicalis* (ATCC 750), and resistant *C. auris* (CDC B11903). The strongest activity in case of bacteria was shown by diethyl ether extract on *E. coli* (ATCC 35210) and *P. aeruginosa* (PAO1) (MICs = 0.25 mg/mL). *C. parapsilosis* (ATCC 22019) was the most susceptible microorganism when treated with all investigated extracts (MIC = 0.125 mg/mL). Also, diethyl ether extract has shown the strongest activity against *C. tropicalis* (ATCC 750) (MIC = 0.125 mg/mL). Ergosterol-binding assay was employed to assess whether diethyl ether extract binds to the fungal membrane sterols of *C. albicans*. The results have shown no changes in MIC value; therefore, the mechanism does not include binding with ergosterol. Further phytochemical and biological activity studies, as well as investigations of the mechanisms of action will help to explain the activity of the strongest extracts.

KEYWORDS: bacteria; bioactivity; *Candida auris*; ergosterol-binding assay; plant extracts

GR116

ANTIMICROBIAL ACTIVITY AND SAFETY ASSESSMENT OF *SCUTELLARIA ALTISSIMA* EXTRACTS

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The occurrence of antibiotic resistance is noted shortly after their discovery and nowadays, it represents a notable health problem. One way to fight this problem is a use of plants' secondary metabolites known as important bioactive compounds. Taking into account all the above, the aim of this study was to investigate the content of phenolic compounds, antimicrobial and antibiofilm activity, as well as to assess selective toxicity of *Scutellaria altissima* 70 % aqueous-ethanolic extracts. The extracts were prepared with plant material collected at three different locations in Serbia (labelled as SaB, SaP, SaS). The highest total phenolic content (79.71 ± 3.11 mg GAE/g DE) and total flavonoid content (39.74 ± 2.60 mg QH/g DE) were recorded for SaS, while SaP was the most abundant in phenolic acids (57.84 ± 0.58 mg CAE/g DE), all determined by colorimetric assays. Results of microdilution assay indicated good antimicrobial effect of extracts towards *L. monocytogenes* (0.156 – 0.3125 mg/mL), *S. flexneri* (0.156 – 1.25 mg/mL) and *C. albicans* (0.156 – 0.625 mg/mL). Further, crystal violet staining of biofilm biomass demonstrated mild antibiofilm effect of all three extracts against *L. monocytogenes* with inhibition of biofilm biomass up to 30 %. In order to test selective toxicity of the extracts, cytotoxicity was evaluated on normal human fetal fibroblasts (MRC-5) using MTT assay, with the survival reduction detected in a range of 27 – 82 %. Additionally, calculation of selective toxicity showed selectivity of SaP and SaS towards microorganisms, specifically *L. monocytogenes*, *S. flexneri*, and *C. albicans*. Obtained results support further investigation and assessment of possible applications of the extracts/pure bioactive constituents as antimicrobial agents.

KEYWORDS: *Scutellaria altissima* ethanol extracts; phenolic compounds content; antimicrobial activity; antibiofilm effect; selective toxicity

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GR117

EFFICACY OF CINNAMON ESSENTIAL OIL AND EMULSION ON *ACINETOBACTER BAUMANNII* COMPONENTS OF BIOFILM MATRIX

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Acinetobacter baumannii is nosocomial pathogen able to form biofilm on different surfaces. Its biofilm is constituted of water, exopolysaccharides, proteins, eDNA, lipids and persister cells. These constituents could be used as a target for antimicrobial agents to disrupt formed biofilm. Cinnamon essential oil (EO) and its emulsion (EM) already exhibited great potential as antibiofilm agents. With that in mind, the aim of study was to further investigate underlying mechanism, i.e. to determine the effect on selected biofilm matrix components, and expression of *aba*I gene being involved in biofilm formation. Bradford method for proteins and phenol-sulphuric acid for exopolysaccharides showed that EO and EM decreased the amount of proteins (inhibitions 75% for EO and 41% for EM), and EO reduced the amount of exopolysaccharides (for 37%). Concentrations of eDNA under the influence of both substances were also lower. In order to provide better understanding of chemical bonds, i.e. molecules presented in biofilm matrix, as well as to further analyze effect of test substances, Raman spectroscopy was applied. The most intensive peak detected in biofilm of all treatments was around 1581 cm^{-1}, which could imply on the presence of nucleic acids (A,G). Principal compound analysis (PCA) of recorded Raman spectra was applied in order to differentiate treatments effects on matrix components. Separation of EM treatment in relation to the treatment of EO and control was visible. Peaks that suggest those differences were at 1308 cm^{-1} and 1653 cm^{-1}, indicating proteins, i.e. amide III group, and saturated lipids, respectively. Further on, RT-PCR was used to determine whether test substances have the impact on *aba*I gene expression. Results showed that after the EM treatment *aba*I expression was decreased, while EO upregulated its expression. Taking all into account it can be concluded that EO and EM exhibited great potential as novel antibiofilm agents against *A. baumannii*.

KEYWORDS: *Acinetobacter baumannii*; biofilm matrix; cinnamon; Raman spectroscopy; gene expression

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PP118

RAPID DETECTION OF BIOFILM-PRODUCING *CANDIDA ALBICANS* VIA MALDI-TOF MASS SPECTROMETRY

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Candida albicans persistence in cystic fibrosis (CF) patients' respiratory tract contributes to lung function decline, primarily due to biofilm formation. Detecting biofilm-producing *Candida* strains is crucial for understanding treatment challenges. This study aimed to assess biofilm formation by *C. albicans* strains from CF patients' respiratory tract and determine the correlation between biofilm mass and specific *C. albicans* proteins using MALDI-TOF MS. Seventeen *C. albicans* sputum isolates from 13 CF patients were evaluated for biofilm formation using safranin staining. MALDI-TOF MS was used to analyse mass spectra, and Pearson's correlation test assessed the relationship between peptide mass intensities and biofilm production. Eleven percent of strains exhibited strong biofilm-producing activity, while 18%, 47%, and 24% were categorized as moderate, weak, and non-biofilm producers, respectively. A strong correlation was found between the intensity of a 6.238 Da protein (± 500 ppm) and biofilm production. A determined cut-off intensity value of 6.848 a.u. distinguished biofilm-producing strains, with intensity higher than 9.977 a.u. indicating strong biofilm producers. Biofilm-producing *Candida* spp. in CF patients pose treatment challenges, with worse prognosis for infected individuals. MALDI-TOF MS analysis revealed differences in peptide mass peak intensities, indicating specific proteins in biofilm-producing *C. albicans* strains. This study highlights the significance of biofilm-producing *C. albicans* strains in CF patients and suggests MALDI-TOF MS as a rapid, cost-effective technique for identifying such strains. Understanding protein composition offers insights for targeted treatment strategies to improve patient outcomes.

KEYWORDS: *Candida*; biofilm; diagnostics; MALDI-TOF MS

PP119

EMPOWERING ANTIFUNGAL DRUGS DISCOVERY THROUGH THE ZEBRAFISH-INFECTIOUS DISEASES MODELLING

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Fungal infections, once considered a rare disease, have become an everyday problem in modern societies, posing major challenges to global health. It is estimated that more than one billion people are affected by fungal infections and 1.6 million people succumb to these diseases every year. Of the 600 species of fungi capable of causing infections in humans, species of the genus *Candida* cause more than 85% of infections, especially *C. albicans*, which has become a serious threat to human health in immunocompromised and immunosuppressed individuals. Unfortunately, the current arsenal of clinical drugs relies on only four classes of approved drugs (polyenes, azoles, echinocandins and allylamines), which are only partially effective, resulting in incomplete eradication of the fungal infection. In addition, the serious side effects, ranging from systemic or organ-specific toxicity to poor bioavailability and low activity, significantly hamper the clinical use of antifungals. These problems call for new effective and safe antifungal agents, but also for appropriate preclinical models to accurately study potential adverse effects on the human population and test their efficacy against fungal infections. In this sense, zebrafish (*Danio rerio*) embryos have become one of the most powerful preclinical animal models in infection biology and drug discovery, offering the unique opportunity to simultaneously monitor the safety and efficacy of the applied molecule in real time. With the aim of providing a preclinical platform for the identification of new safe antifungal drugs to effectively control *C. albicans* infection, we comprehensively tested the toxicity of 13 clinical antifungal drugs in the zebrafish embryo model. The 21 toxicity endpoints, including survival, teratogenicity, cardiotoxicity and hepatotoxicity, were evaluated and compared with adverse effects described in rats and humans. Of the clinical drugs, the efficacy of fluconazole and voriconazole was evaluated in the zebrafish - *C. albicans* model of systemic and wound biofilm infection.

KEYWORDS: zebrafish model; antifungal drugs; *Candida albicans*; infection modelling

PP120

THE ANTIADHESIVE ACTIVITY OF NITROXOLINE AND HYDROQUINONE TOWARDS UROPATHOGENIC ENTEROCOCCUS ISOLATES AS A NATURAL APPROACH IN THE CONTROL OF URINARY TRACT INFECTION

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Bacterial adherence either to host tissues or biomaterials is the first step in the colonization of these surfaces allowing bacteria to form biofilms in which they are protected from harmful environmental factors. *E. faecalis* has a high level of antibiotic resistance, and forms robust biofilms wich is factor that contributes to *E. faecalis* infection, colonisation and antibiotic resistance. Nitroxoline appears to be an alternative to other uroantiseptics owing to its favourable safety profile, however data on its current in vitro susceptibility are sparse. Hydroquinone is recognized as an active substance at the site of action (urinary tract) and it might be important for the therapeutic activity of a herbal preparation. The goal of the following experiment is to estimate the minimum concentration of hydroquinone and nitroxoline that is needed to inhibit and kill the enterococci, as well as to assess the effect of sinergy against adherent enterococci. Antibacterial and antibiofilm activity through a synergistic effect of nitroxoline and hydroquinone was evaluated to discover an alternative approach to controlling urinary tract infections caused by *Enterococci*. Among the 35 uropathogenic isolates of enterococci tested had a nitroxoline MIC range of 4-16 µg/ml and hydroquinone MIC range of 0,78-1,2 mg/ml. The individual effect of nitroxoline and hydroquinone on adhesion was examined, where the antiadhesive effect is shown only by nitroxoline, while hydroquinone did not show this examined effect. Using a checkerboard assay, the synergistic antiadhesive effect of hydroquinone and nitroxolin on the examined enterococci in different combinations was detected. The achieved synergistic effect of the tested combinations might be a starting point in the effective treatment of antibiotic resistant enterococcus infections. Further experimental studies should be performed to elucidate this point.

KEYWORDS: *enterococcus*; urinary tract infection; antiadhesion

PP121

DRUG REPURPOSING TO INHIBIT *PSEUDOMONAS AERUGINOSA* ADAPTATION TO THE CF LUNG ENVIRONMENT

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The success of a bacterial pathogen in establishing hard-to-eradicate infections strictly correlates to its ability to use the host environment as a growth medium, and to produce virulence factors and resists to the action of antibiotics inside the host. The opportunistic human pathogen *Pseudomonas aeruginosa* uses the airway sputum as a nutritional source during cystic fibrosis (CF) lung infection, and finely modulates the formation of antibiotic-resistant biofilms and virulence factors production in response to stimuli associated to the host environment through c-di-GMP and quorum sensing (QS) signaling systems. The CF sputum has been characterized and reconstituted as a synthetic CF sputum medium (SCFM). Genes required for *P. aeruginosa* growth in SCFM are dispensable in standard laboratory media. Moreover, *P. aeruginosa* displays similar biofilm formation and QS activation during growth in the CF sputum and in SCFM. Hence, unexplored molecular pathways are required for growth, virulence and biofilm formation in the CF sputum, and molecules inhibiting these pathways in SCFM have the potential to reduce *P. aeruginosa* load and pathogenicity in the CF lung. To identify these molecules, we will screen a library of FDA-approved drugs using an *ad hoc* engineered biosensor strain cultured in SCFM. Here, we will present the generation and validation of a *P. aeruginosa*-based biosensor strain in which molecules hampering c-di-GMP or QS signaling systems decrease light or fluorescence emission, respectively. Interestingly, the known c-di-GMP and QS inhibitors sodium nitroprusside and niclosamide were more effective in reducing the biosensor activity in the standard medium Mueller Hinton Broth (MHB) than in SCFM, highlighting the necessity to identify new drugs that specifically inhibit *P. aeruginosa* biofilm formation and virulence in SCFM. Preliminary data collected during the screening of a library of more that 3,000 FDA-approved drugs using the biosensor strain grown in SCFM will be also presented.

KEYWORDS: *Pseudomonas aeruginosa*; cystic fibrosis; new antimicrobials; quorum sensing; c-di-GMP

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PP122

YTNP LACTONASE IMPROVES THE ABILITY OF *CAENORHABDITIS ELEGANS* TO SURVIVE *PSEUDOMONAS AERUGINOSA* MMA83 INFECTION

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Pseudomonas aeruginosa is a Gram-negative pathogen responsible for frequent hospital-acquired infections of the bloodstream, the respiratory tract, and the urinary tract. Quorum quenching enzymes are recognized as an alternative antivirulence approach targeting pathogenic bacteria. The efficacy of YtnP lactonase in reducing the virulence of *P. aeruginosa* MMA83 *in vivo* using *Caenorhabditis elegans* as a model system was investigated. The recombinant YtnP lactonase exhibits no cytotoxicity, demonstrated by its lack of harmful effects on both the immortalized human HaCaT cell line and two strains of *C. elegans* (AU37 and N2 wild-type). In a toxin-mediated killing liquid assay, the survival rates of *C. elegans* AU37 mutant and N2 wild-type strains infected with the clinical isolate *P. aeruginosa* MMA83 significantly increased when pre-treated with YtnP lactonase, compared to untreated controls. Considering that virulence factors expression is regulated by quorum sensing (QS) signaling it is hypothesized that YtnP lactonase prolongs the life span of *C. elegans* by downregulating the QS and expression of virulence factors of MMA83. The protective effects of YtnP lactonase against MMA83-induced pathogenicity in *C. elegans*, coupled with its absence of cytotoxicity, position YtnP lactonase as a promising prophylactic agent with antivirulence properties.

KEYWORDS: *C. elegans;* lactonase; *P. aeruginosa;* infection model

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PP123

EFFICACY OF APPLICATION GAS AND LIQUID DISINFECTANT FOR INHIBITION OF MICROBIAL GROWTH

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Throughout human history, a symbiotic relationship has existed between humans and microorganisms, characterized by a perpetual struggle. As our understanding of microorganisms has grown, so too has our ability to foster mutualistic or commensal relationships and enhance our resistance. However, the emergence of infectious diseases has cast a dark shadow over human existence, with microorganisms evolving efficient transmission mechanisms to propagate and inflict harm. The advancement of scientific knowledge has facilitated rapid responses to potential pandemics, as witnessed with the emergence of the SARS-CoV-2 virus, causing COVID-19. This study aimed to test the efficacy of inhibiting microorganism growth using different concentrations of ethanol (70% and 96%), hydrogen peroxide (3% and 6%), and ozone (2.5 ppm). Disinfection of frequently touched items, such as doorknobs, phones, keyboards, and backpacks, was also evaluated. The diffusion method assessed antimicrobial activity, with *Escherichia coli*, *Bacillus subtilis*, *Aspergillus niger*, *Candida albicans*, and *Saccharomyces cerevisiae* used as control strains. Hydrogen peroxide at 3% and 6% concentrations effectively prevented the growth of fungi, yeasts, molds, and bacteria on all tested surfaces. Both 70% and 96% ethanol concentrations inhibited fungi, yeasts, and molds, with 70% ethanol exhibiting better results against bacteria. Ozone treatment for 5 and 10 minutes reduced the presence of all microorganisms. Comparing the results, 6% ozone concentration demonstrated the best outcome, while 70% ethanol showed relatively weaker results. Ozone for 10 minutes effectively removed microorganisms from surfaces to a greater extent.

KEYWORDS: antimicrobial activity; ethanol; hydrogen peroxide; ozone

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PP124

MEDICINAL MUSHROOM EXTRACTS ATTENUATE *PSEUDOMONAS AERUGINOSA* QUORUM SENSING AND VIRULENCE

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Pseudomonas aeruginosa has been recognized as a priority pathogen by World Health Organization, due to the emergence of multidrug-resistant (MDR) strains. Thus, new treatment options such as antivirulence strategy is urgently needed. This strategy is based on the disruption of quorum sensing (QS) activity of this pathogen. The focus of this research was to explore the anti-QS activity of four selected medicinal mushrooms (*Lentinula edodes*, *Cantharellus cibarius*, *Trametes versicolor* and *Pleurotus ostreatus*) extracts on MDR clinical isolate *P. aeruginosa* MMA83. Another aim was to check their cytotoxicity on *Caenorhabditis elegans* AU37 (glp-4(bn2) I; sec-1(km4). Among three types of mushroom extracts - hot water polysaccharide extracts (WPE), hot alkali polysaccharide extracts (APE) and methanol extracts (Met), APE extracts downregulated all tested QS and virulence factors genes of *P. aeruginosa* MMA83. The most prominent effect was observed for *C. cibarius* APE extract, lowering expression from 2-fold (for *lasI* gene) to 20-fold for *lasB* gene. Extracts didn't show cytotoxic effect on *C. elegans*. The efficacy of APE extracts in lowering the expression of QS and virulence factors genes of *P. aeruginosa* MMA83 indicate that these extracts can reduce pathogenicity of *P. aeruginosa*. Also, they possess one of the desirable biotechnology features – the absence of cytotoxicity. Anti-QS and antivirulence effect of APE extracts on *P. aeruginosa* envisages these extracts as the promising therapeutic candidates for the development of next-generation antivirulence agents.

KEYWORDS: medicinal mushroom extracts; *Pseudomonas aeruginosa*; quorum sensing inhibition; antivirulence

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PP125

ACTIVATED CHARCOAL AS A CARRIER OF PROBIOTICS: A NEW APPROACH FOR PATHOGEN ELIMINATION IN WOUNDS - PROHEALINGAC

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According to World Health Organization (WHO), antibiotic resistance is one of the biggest threats to global health, food security and development today. However, development of conventional anti-infective drugs is going slowly, so new innovative strategies and more research are urgently needed in developing, implementing and evaluating novel therapies for antibiotic-resistant infections. The two-year ProHealingAC project, funded by the Science Fund of the Republic of Serbia, aims to use beneficial properties of activated charcoal (AC) and probiotic microorganisms in developing the new strategy for prevention and local treatment of antibiotic-resistant infections in wounds. Precisely, the aim is to develop efficient, simple and cost-effective biocomposites (BCs) based on AC fabric (ACF) and probiotics in order to achieve their synergetic activity for efficient and sustained local delivery of bioactive agents directly into the wound area. Also, special attention has been given to the influence of glucose level (normo- and hyperglycemia) in the microenvironment of the wound. Developed BCs will be comprehensively characterized *in vitro* regarding probiotic release profile, antimicrobial and antibiofilm activity as well as modulation of macrophage, fibroblast and keratinocyte activity. Based on the obtained results, the best candidate will be selected for in vivo studies in wound model in diabetic and non-diabetic animals. Furthermore, through efficient dissemination and communication of the results, ProHealingAC project will help raise people's awareness of the importance of the rational use of antibiotics which is crucially important since the Republic of Serbia is one of the countries with a high rate of resistance in both human and veterinary medicine as well.

KEYWORDS: antibiotic resistance; local delivery; topical therapy; biocomposites; biofilm; diabetes

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PP126

CHEMICAL COMPOSITION AND QUORUM SENSING INHIBITION ACTIVITY OF HORSERADISH (*ARMORACIA RUSTICANA*) ROOT EXTRACTS

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During the past decades several quorum sensing inhibitors (QSI) of plant origin have been isolated and chemically characterized. QSI agents of plant origin represent potential alternative or complementary approach to antibiotic treatment of multidrug-resistant bacteria and infections caused by bacterial biofilms. The aim of the current study was to screen QSI activities of horseradish root extracts obtained using different organic solvents and different root processing methods (drying at 40°C, 60°C or extraction of fresh material). Common opportunistic pathogen *Pseudomonas aeruginosa* MMA83 was used for QSI screen. RT-qPCR was used to analyze the effect of the extract on the relative mRNA levels of the genes QS (*lasR, lasI, rhlR, rhlI, mvfR, pqsH*) and the genes involved in *P. aeruginosa* MMA83 virulence (*lasB, phzM, rhlC, algK, pvdS*). Chemical composition of extracts was determined by UHPLC Q-ToF MS analysis. The most active extract obtained using fresh roots and hexane/ethyl acetate (1:1) solvent mixture was able to significantly reduce content all examined mRNA. Qualitative chemical analysis reviled presence of 15 phenolic acids and their derivatives, 9 flavonoids and 10 glucosinolates in majority of examined extracts. It is significant to emphasize that the most active QSI extract did not contain a single one, out of ten dominant glucosinolates, which have undergone to hydrolysis yielding isothiocyanates and other sulphur-containing compounds responsible for QSI effects. Our results strongly indicate that even mild thermal treatment (40°C) of horseradish roots prior to extraction could lead to severe reduction or loss of QSI activity.

KEYWORDS: horseradish; *Pseudomonas aeruginosa;* quorum sensing inhibition; UHPLC Q-ToF MS

ACKNOWLEDGEMENT: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreement no - 451-03-47/2023-01/ 200042).

PP127

NOVEL BACTERIOPHAGE ISOLATION FROM BELGRADE WASTEWATERS

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Anti-microbial drug resistance (AMR) is one of the global health threats caused by the misuse of drugs typically used to treat microbial infections in humans, animals and plants. AMR in nosocomial infections not only significantly hinders treatment and endangers the patients' lives, but also elevates the costs of healthcare. Multiple research approaches have been initiated to combat AMR, and one promising method is bacteriophage therapy. Bacteriophages (phages) are viruses that naturally exploit bacteria as their hosts for replication and can cause cell lysis, which makes them promising candidates for treating the infections that do not respond to conventional antibiotic therapies. In this study, we screened wastewater samples from four different collectors in Belgrade urban area for bacteriophages active against clinically isolated strains of two biofilm-producing bacteria that readily persist in hospital environment - *Klebsiella pneumoniae* (6 strains) and *Pseudomonas aeruginosa* (2 strains). Wastewaters were screened for phage presence through phage enrichment process, in which bacteria were grown in a mixture of water samples and nutrient-rich broth. Obtained cultures were screened for antimicrobial activity against the respective host strains, and candidates were subjected to a first-round plaque assay to detect the phages. Finally, the activity of all the candidates was tested against all strains of the same species to gain the first insight into their host range. We discovered 20 potentially distinct bacteriophages active against *K. pneumoniae* strains and two potentially different candidates targeting *P. aeruginosa*. Notably, one phage exhibited activity against all tested *K. pneumoniae* strains, and four were active against 5 out of 6 tested strains. Among 22 candidates in total, five showed depolymerizing activity, indicating promise in combating biofilm formation. Currently, isolation of new phages, as well as purification and host range analysis is underway for several candidates targeting *K. pneumoniae* and two targeting *P. aeruginosa* strains.

KEYWORDS: bacteriophage isolation; phage therapy; enrichment culture; anti-microbial drug resistance (AMR)

PP128

ANTIMICROBIAL POTENTIAL OF RESVERATROL NANOBELT-LIKE PARTICLES

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The emergence of microbial resistance to commonly used antibiotics has induced search for novel antimicrobial agents. As natural sources have already provided many of the conventional drugs, they can also be a basis for new ones. Resveratrol is a stilbenoid polyphenol, synthesized by plants as a protective compound. Among its' many biological activities, antimicrobial have often been reported. On the other hand, there are several obstacles for the practical use of this compound, among them solubility and general difficulty of efficient delivery. Nanotechnology has allowed recent progress regarding the use of many natural compounds. However, it usually relies on the use of additional expensive or toxic compounds during the synthesis of nanoparticles. In our research, we used simple, green synthesis to prepare nanoparticles of pure resveratrol, in a nanobelt-like form. Shape, uniform size and absence of carrier substances made these particles convenient for the implementation of resveratrol for different purposes. We tested their potential to inhibit the growth of several bacterial strains, reference and clinical, and one micromycetes strain. For the determination of antibacterial effect, we used microdilution method followed by optical density measurements, resazurin staining and agar plating, to determine effect on growth, as well as minimal inhibitory and minimal bactericidal concentration. In case of microfungal cultures, we used MTT staining for the testing. There were significant differences in the effects, between gram-positive and gram-negative bacteria, and microfungi. While the concentration that led to the inhibition of growth of *Staphylococci* was 800 µg/ml, the growth of gram-negative bacteria was inhibited only at several times higher concentrations. *Aspergillus caespitosus* showed significantly higher sensitivity, with MIC/MBC being 200 µg/ml. These results indicated possible use of these particles in further biomaterial design as an additive component with moderate antimicrobial activity.

KEYWORDS: resveratrol; nanoparticles; antimicrobial; polyphenol

ACKNOWLEDGEMENT: Funds for the realization of this work were provided by the Ministry of Science, Technological Development and Innovation (MoSTDI) of the Republic of Serbia, Agreement on realization and financing of scientific research work of the Institute of Technical Sciences of SASA in 2023 (Record number: 451-03-47/2023-01/200175) and Grant No: 451-03-47/2023-01/200178.

PP129

ANTIBIOFILM ACTIVITY OF *ACHILLEA MILLEFOLIUM* EXTRACTS

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Bacterial biofilms are considered an important virulence factor that causes persistent chronic and recurrent infections. Plant extracts and their active compounds have a significant role in the prevention and treatment of diseases since ancient times. In this study, the *in vitro* antibiofilm activity of acetone, ethyl acetate, and ethanol extract of *Achillea millefolium* was investigated using a crystal violet assay. Further, the effect of the extracts on auto-aggregation, motility and cell hydrophobicity was evaluated. *A. millefolium* extracts showed their strong antibiofilm activity against strains isolated from human wounds. The extracts of *A. millefolium* significantly inhibited initial cell attachment and biofilm formation, but the reduction of established biofilm was weaker. *A. millefolium* extracts had dose- and strain-dependent effects. The antibiofilm rates rise with an increase in extract concentration. In addition, Gram-positive bacterial strains were more sensitive than Gram-negative strains. Furthermore, *A. millefolium* extracts reduced bacterial auto-aggregation but did not disturb bacterial cell hydrophobicity. Swimming and swarming motility of some strains were also reduced in the presence of the extracts. Our study revealed the potential role of *A. millefolium* extracts as a new antibiofilm agent against human pathogenic bacteria. Therefore, isolation and identification of natural constituents that exhibit antibiofilm activity might be good alternatives in the control of biofilms.

KEYWORDS: Bacterial biofilm; pathogenic bacteria; plant extract; *in vitro* testing

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PP130

CITRUS FLAVONOIDS AS INHIBITORS OF VIRULENCE TRAITS IN *CANDIDA SPP.*

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Citruses are known for a range of beneficial properties to human health, partly due to high levels of flavonoids present in them. Citrus flavonoids have numerous bioactivities, but their antivirulence potential is not yet fully elucidated. The aim of this study was to determine effect of three flavonoids: taxifolin, hesperetin, and sakuranetin on growth and virulence of *Candida* species, as well as to screen for their toxicity. They inhibited growth of *C. albicans* (strains 10/15, 13/15, ATCC 10231, 475/15), *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750, *C. krusei* H1/16, and *C. glabrata* 4/6/15 with MICs ranging 0.041 – 0.165 mg/mL as determined by microdilution assay. They have reduced ability of *C. parapsilosis* ATCC 22019 to group into biofilm (over 60% of biofilm biomass inhibition). Moreover, they were also able to diminish pre-established biofilms (~50 % and more elimination in case of *C. albicans* 475/15). Their mechanism of antibiofilm activity is partly based on the reduction of exopolysaccharide level in the biofilm matrix. Since the cytotoxicity study on MRC-5 cells indicated that hesperetin was the least toxic among them (at a concentration 100 µg/mL induced 78.6% cell survival, compared to 70.3% and 9.5% for taxifolin and sakuranetin, respectively), it was selected as the lead molecule for further studies. Screening of hesperetin effect on polymicrobial *C. albicans* ATCC 10231 and *Staphylococcus aureus* ATCC 11632 biofilm showed inhibitory potential, but at high concentrations (1 mg/mL was necessary for > 50% reduction in cell viability and > 40% reduction in biofilm biomass). Brine shrimp lethality assay further confirmed safety of hesperetin with LC50 > 1 mg/mL. Although hesperetin exhibited significant antifungal potential, its activity against polymicrobial biofilm is achieved at high concentrations. Citrus flavonoids have unique traits and further research is necessary to enlighten their potential, primarily antivirulence activity.

KEYWORDS: citrus flavonoids; virulence; antimicrobials; *Candida* spp.

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ACTIVE IMMUNIZATION AS THE KEY ELEMENT IN INFECTION PREVENTION AND CONTROL

IL131

MMR VACCINE AND SEROPREVALENCE OF MEASLES, MUMPS AND RUBELLA IGG ANTIBODIES AMONG YOUNG MEDICAL STUDENTS IN SERBIA

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Measles, mumps and rubella are vaccine-preventable diseases caused by viruses. They are most common in children and young adults and can lead to potentially complications and death. All of them are transmitted via the respiratory route and present a high occupational risk for healthcare workers and patients with whom they come in contact. Measles is a highly contagious disease and a leading cause of death among children below five years-old worldwide. Although measles usually has a simple course, severe complications can occur, including encephalitis, SSPE and death. Rubella, in non-immunized pregnant women, may pose a high risk of fatal complications. The MMR vaccine is a combined vaccine. One dose of the measles vaccine is 95% effective, and two doses are 96% effective in preventing measles. In order to eliminate measles, it is necessary to reach and maintain measles vaccination coverage at 95%. Achieving mumps elimination is considered difficult due to potential Mumps virus importations, and the current two doses of vaccination are "only" 86% effective against diseases. According to data, one dose of MMR vaccine is 89% effective in preventing rubella. Levels of Mumps antibodies decline over time, and immunity may persist for 10-15 years. Rubella immunity is considered lifelong, but studies have shown that immunity lasts only 15 years. In Europe, a drop in vaccine coverage rates to suboptimal levels has been reported in recent years. The preliminary results of the study that is still ongoing at the Institute of Microbiology and Immunology MFUB among 600 medical students showed that protective anti-measles IgG, anti-mumps IgG, and anti-rubella IgG titers were 74%, 73% and 81%, respectively. The management of non-responder healthcare workers with respect to the MMR vaccine must be considered in future decisions on vaccination strategies.

KEYWORDS: MMR vaccine; IgG antibodies; seroprevalence; medical students

IL132

ADVANCES, CHALLENGES AND NOVELTIES IN HBV AND HCV VACCINE DEVELOPMENT

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Infections with the hepatitis B virus (HBV) and hepatitis C virus (HCV) remain a global health problem, with an estimated 296 million and 71 million chronically infected individuals, respectively. Generations of vaccines against HBV infection, based on hepatitis B surface proteins, were developed over time and have excellent protection rates. For a small proportion of non-responders in high-risk groups, more efficient vaccine variations were approved recently and the success of prophylactic vaccination inspired research in therapeutic anti-HBV immunisation as well. Highly effective direct-acting antivirals (DAAs) have revolutionised the treatment of chronic hepatitis C, eliminating the infection during short treatment protocols and without adverse effects. However, several reasons make the development of a preventive vaccine necessary: it is estimated that the HCV epidemic is still growing, screening of chronic carriers for antiviral treatment is complex and most infected individuals are not identified, treatment costs remain high, and re-infections of persons at risk are common. Challenges to developing efficient HCV vaccines are still not overcome, including virus genetic variability, limited models for testing vaccines, and incomplete knowledge of protective immunity. Antigen candidates for the HCV vaccine included envelope, core and non-structural proteins. The vaccine types tested were protein-based, DNA-based, VLP-based, pox- and adenovirus-vector-based, and whole-virus-based. It is conceivable that the success of the first mRNA-based vaccine against SARS-CoV-2 would bring a new strategy in HCV vaccine development.

KEYWORDS: hepatitis B virus (HBV); hepatitis C virus (HCV); vaccine development

IL133

VACCINATION AGAINST PERTUSSIS IN SERBIA: PAST, CURRENT CHALLENGES AND FUTURE PERSPECTIVES

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Vaccination against pertussis is a long-standing preventive strategy in Serbia that has influenced reduction of pertussis incidence and mortality in the past, especially among infants. Whole-cell pertussis vaccine, as a part of the combined vaccine against diphtheria, tetanus and pertussis (DTP), was introduced in Serbia in 1957 and was used until 2015, when it was replaced by acellular pertussis vaccine. Acellular pertussis vaccine contains only a few specific antigens and is less reactogenic, but induces immunity of a shorter duration compared to the whole-cell vaccine. According to the current National mandatory immunisation schedule acellular pertussis vaccine, as a part of combined vaccines, is given as a primary series of three doses between 2nd and 6th month of age, followed with two booster doses during the 2nd year of age and before elementary school entry. During 2023 a resurgence of pertussis was registered in Serbia with 1349 notified confirmed cases and three pertussis-related deaths in unvaccinated infants younger than three months. The most cases and the highest age-specific incidence rate were registered among adolescents aged 10 to 14 years. Potential factors that have influenced the increase of number of notified pertussis cases are: waning of vaccine induced immunity, decline in immunization coverage, asymptomatic transmission of Bordetella pertussis in adolescents and adults, emergence of new Bordetella pertussis strains different of those contained in the current vaccines as well as increased awareness of the disease and more available laboratory diagnostic. Future strategies for pertussis control in Serbia will include the introduction of an additional booster dose of acellular pertussis vaccine during the last grade od elementary school in the National mandatory immunisation schedule, mandatory immunisation of health care workers and recommended immunisation during pregnancy.

KEYWORDS: pertussis; vaccination; Serbia

IL134

DO WE NEED HIGHER VALENCY PNEUMOCOCCAL CONJUGATE VACCINES IN SERBIA?

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In Serbia the 10-valent pneumococcal conjugate vaccine (PCV10) was introduced in the National Immunization Programme (NIP) for children younger than 2 years in 2018. It was replaced by PCV13 in 2022, but in 2024, PCV10 is returned in the NIP. Currently, four PCVs are in use worldwide: PCV10, PCV13, PCV15, and PCV20, with the first three being registered in Serbia. Continuous surveillance of invasive pneumococcal disese (IPD) is crucial to understand local serotype distribution and to provide essential information to support PCV immunisation policy in the country. Based on the data of the National Reference laboratory (NRL) for streptococci, before 2018, the prevalent serotypes across all age groups were 3, 19F, 14, 6B, 6A, 19A, and 23F. Among children under two years, the most common serotypes included 19F (18.8%), 14 (17.8%), 6B (15.8%), and 6A (8.9%). PCV10 and PCV13 serotypes coverage among children younger than two years was 71.3% and 86.1%, respectively. In the post-2018 period, a gradual shift in serotype distribution occurred, notably among children. Serotypes 14 and 19F nearly vanished, while the frequency of 6A and 6B decreased by approximately threefold in children under 2 years. Conversely, there was a notable surge in serotypes 19A (from 3.2% pre-2018 to 22.2% in 2022/2023) and 3 (from 4.2% pre-2018 to 14.8% in 2022/2023). By 2023, PCV10 coverage among pediatric populations was low, only 21.6%, while for PCV13 and PCV15 it was 64.9%, and 73%, respectively. Drawing from Belgium's experience, where the replacement of PCV13 with a lower-valent PCV10 resulted in a rise in IPD incidence and a dramatic increase in serotype 19A frequency, it's highly likely that Serbia may face a similar scenario. Given the low coverage of the PCV10 vaccine, it is imperative to swiftly introduce a multi-valent PCV – PCV13 or PCV15, which are registred in our country.

KEYWORDS: pneumococcal conjugate vaccine; serotype; children; Serbia

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IL135

MRNA VACCINE MANUFACTURING – CHALLENGES IN PLASMID DNA CLONING VECTOR DESIGN

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In the post-COVID-19 era, there has been a significant increase in the development of mRNA vaccines not only against various diseases besides SARS-CoV-2, but also to treat cancer and genetic disorders. These vaccines, revolutionizing vaccinology, offer rapid pandemic response, high efficacy, minimal side effects, and cost-effectiveness. Achieving these benefits hinges on seamlessly integrating mRNA production steps, from plasmid DNA cloning to lipid nanoparticle formulation. This overview aims to comprehend or circumvent pitfalls in plasmid DNA cloning, a critical initial step in mRNA vaccine production. The focus is on achieving accurate insert sequence and gene expression, and it highlights the critical role of plasmid DNA design in ensuring vaccine effectiveness. Our research project entitled "Role of macroautophagy in lipid nanoparticle mRNA delivery and adjuvanticity" recognized the significance of this aspect. During our research, we designed a plasmid DNA cloning vector to incorporate the GFP-SARS-CoV-2 Spike gene. The vector was carefully constructed with several key features, including a high-copy plasmid, pUC18/pUC19 vector backbone with a robust T7 promoter, origin of replication, multiple cloning sites, polyadenylation signal, and ampicillin resistance for bacterial selection. Despite careful design, challenges like poly-A tail deletion may arise, prompting the exploration of stable large-size and low-copy vectors, as well as linear and bacteriophage vectors. But, for large-scale production and regulatory compliance, vector systems must be scalable and well-documented. Commercial vectors and automated synthesis facilitate gene construction, with artificial intelligence ensuring sequence accuracy. Precision is crucial for complex antigens, as seen in tuberculosis mRNA vaccine development. Addressing these challenges demands a combining of molecular biology techniques, computational tools, and collaboration with experts in microbiology, molecular biology, and vaccine development. The design's scalability and documentation are vital for large-scale production and regulatory compliance, emphasizing the multifaceted approach required for successful mRNA vaccine development.

KEYWORDS: mRNA vaccine; plasmid DNA cloning vector design

ACKNOWLEDGEMENT: in lipid nanoparticle mRNA delivery and adjuvanticity – REDIRECT This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 11132, Role of macroautophagy.

IL136

DEVELOPMENT OF NEW VIRAL VACCINES WITH A FOCUS ON THE NEW RSV VACCINE

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Human respiratory syncytial virus (RSV) is the most common viral pathogen identified in children with acute respiratory tract infections (ARTIs). Natural infection does not provide long-lasting immunity and reinfections with RSV occur throughout life. The overall burden of RSV includes need for hospitalizations, greater mortality in specific populations and regions (e.g., elderly, developing countries), and medically attended lower respiratory illnesses. Despite numerous studies documenting the significant impact of RSV in infants, young children, the elderly, and other groups with chronic medical conditions, management remains primarily supportive. It includes the administration of supplemental oxygen, adequate hydration, and mechanical ventilation when needed. RSV infection affects an estimated 64 million people leading to 160000 deaths worldwide each year. Before 2019, in 2015, the modelling study estimated that 33 million episodes of RSV-associated acute respiratory infection globally resulted in about 3 million hospital admissions and almost 60,000 in-hospital deaths in children younger than five years. About 45% of hospital admissions and in-hospital deaths were due to RSV in children younger than six months. Thus, an effective RSV vaccine or monoclonal antibody for both pregnant women and new-borns could substantially affect disease incidence in this age population. RSV vaccine development began in the 1960s. In recent years, increased understanding of the biology of RSV and associated technological advances have resulted in the entry of multiple vaccine candidates into clinical development. In 2023, U.S. Food and Drug Administration approved Arexvy, the first respiratory syncytial virus (RSV) vaccine it is approved for the prevention of lower respiratory tract disease caused by RSV in individuals 60 years of age and older. The implementation strategy of new RSV vaccines represents a priority in the upcoming period following tremendous progress in RSV vaccines and therapeutics in the last years.

KEYWORDS: RSV; vaccine; acute respiratory tract infections

IL137

RESPIRATORY INFECTIONS IN CHILDREN – OVER THE HORIZON

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Acute lower respiratory diseases – bronchiolitis, and pneumonia are leading causes of death in childhood in developing countries. Immunization, early diagnosis and proper treatment resulted in a decrease in mortality rate. Passive immunization against Respiratory Syncytial Virus (RSV) had a significant impact on the prevalence of RSV bronchiolitis among high-risk groups of infants. The recent approval of the RSV vaccine for the general newborn population will hopefully reduce its prevalence even more. In the last two years in the Mother and Child Health Institute of Serbia, almost 1200 patients were hospitalized for bronchiolitis, with RSV as the most prevalent causative agent. Parapneumonic effusion and necrotizing pneumonia are severe complications of community-acquired pneumonia in children, despite the introduction of pneumococcal conjugate vaccine (PCV) in the majority of vaccination schedules in European countries and worldwide. The clinical course is fulminant with the development of severe life-threatening complications. Just in the last two years, 360 patients were hospitalized for pneumonia. The complicated clinical course had 78 patients that received PCV in 68% of cases – 75 (21,9%) parapneumonic effusion, 26 (7,6%) necrotizing pneumonia and 5 (1,6%) lung abscess. An outbreak of pertussis infection in Belgrade and Serbia in late 2023 resulted in 46 hospitalized, mostly non-vaccinated infants with a median age of 2.5 months. Six of them had acute respiratory insufficiency and four children died. There is an emerging need for careful updates of the vaccination schedule and an increase in number of vaccinated children.

KEYWORDS: childhood respiratory diseases; RSV immunization; pneumonia complications; pertussis outbreak

IL138

HPV VACCINES IN THE CANCER PREVENTION – RECOMMENDATIONS AND FUTURE PROSPECTIVE

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The causative association between Human papillomaviruses (HPV) and cervical cancer has been well established. In August 2020, WHO has adopted the global strategy to eliminate cervical cancer where 90-70-90 targets that must be met by 2030 are proposed: 90% of HPV vaccinated girls by the age of 15; 70% of screened women by 35 and 45 years of age and 90% of treated women with identified cervical disease. The HPV L1 protein, assembled into virus-like particles (VLPs), induces HPV-type-specific neutralising antibodies, forms the basis of all commercial HPV vaccines. Up to date, six prophylactic HPV vaccines have been licensed for use: three bivalent, two quadrivalent vaccines and one nonavalent vaccine. Current evidence suggests that all of these vaccines offer comparable immunogenicity, efficacy and effectiveness for the prevention of cervical precancer and cancer, which is mainly caused by HPV types 16 and 18. The key element in the primary prevention is implementation of HPV vaccination in national immunization programmes. The choice of HPV vaccine should be based on an assessment of country relevant data such as the incidence of cervical cancer and genital HPV infection. Newest data have demonstrated that a single-dose HPV vaccine protects against HPV at a level comparable to two-dose schedules. The WHO now recommends single-dose schedule for girls aged 9–14 years and 15-20 years, while two-dose schedules are recommended for women older than 21 years. HPV vaccines can be co-administrated with other vaccines, but interchangeable use of HPV vaccines is not recommended. It should be pointed out that HPV vaccination does not eliminate the need for cervical cancer screening later in life, since the existing vaccines do not protect against all high-risk HPV types and will have limited impact on disease in unvaccinated women and those vaccinated at older ages.

KEYWORDS: cervical cancer; Human papillomavirus; prevention, vaccine

IL139

VACCINES FOR INFLUENZA AND COVID-19 – WHAT WE NEED TO KNOW

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Multiple flu viruses usually circulate during one season. As such, the seasonal flu vaccine protects against three or four different flu viruses. These are usually an influenza A (H1N1 and H3N2) viruses, and one or two influenza B viruses in the vaccine. Which influenza viruses the vaccines protect against is decided by research and surveillance of which flu viruse will be the most common during the upcoming season. Flu viruses are constantly changing so it is not possible to predict this with certainty. The flu vaccine effectiveness can vary, and it reduces the risk of flu from 40-60%. The effectiveness depends on the age and health status of the vaccine recipient and the degree of match between the vaccine and the flu strain encountered. When one or more of the circulating viruses are different from the vaccine viruses, vaccine effectiveness is reduced. The COVID-19 vaccine is targeting SARS-CoV-2. Similar to the flu vaccine, it is expect updated covid vaccines to protect against the expected strains of the SARS-CoV-2 that season. Also, the updated vaccines are expected to provide better protection against COVID-19 than the currently circulating variants. The bivalent vaccines target both the original SARS CoV-2 and the two most recent Omicron subvariants (BA.4 and BA.5), which are more contagious than earlier strains. Current COVID-19 vaccines provide strong protection against moderate to severe illness caused by most variants, and are likely to provide at least reasonable protection against others. Because SARS-CoV-2 mutates more slowly than influenza, vaccines may need to be updated less frequently. And finally, it will be easier and faster to modify new mRNA and vectored SARS-CoV-2 vaccines than traditional influenza vaccines.

KEYWORDS: Covid-19; SARS-CoV-2; vaccines; influenza

IL140

POLIOVIRUS ERADICATION: CHALLENGES OF CONTAINMENT

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Two of three strains of wild poliovirus have been declared globally eradicated. In September 2015, the Global Commission for the Certification of Eradication of Poliomyelitis declared wild poliovirus type 2 as eradicated, and in October 2019, wild poliovirus type 3 followed. However, wild poliovirus (WPV1) and circulating vaccine derived polioviruses (cVDPV) cases continue to be registered, albeit with declining number of cases and more focused geographic concentration. Furthermore, with continuing use of type 2 or type 3-containing oral polio vaccines across the world for outbreak response or routine immunization, a number of facilities worldwide, still handle or store the viruses for activities such as vaccine production, polio diagnostics and research. Hence, under the International Health Regulations, the risk of international spread of poliovirus remains a Public Health Emergency of International Concern (PHEI), in the context of the global target of eradication of WPV and cessation of outbreaks of cVDPV2.This points to the importance of Containment, as the key objective of the GPEI's Polio Eradication Strategy 2022-2026, and will be critical for achieving and maintaining a polio-free world. Containment includes biosafety and biosecurity requirements for laboratories, vaccine production sites, or any other facility that handles or stores eradicated polioviruses, to minimize the risk of these viruses being released into the community. Containment also concerns risk mitigation measures associated with field use of some live oral polio vaccines.

KEYWORDS: poliovirus; eradication; containment

IL141

COMPLICATIONS OF PNEUMOCOCCAL PNEUMONIA IN CHILDREN

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Complicated pneumonia, in a previously healthy child, is a severe disease characterized by a combination of local complications (eg, parapneumonic effusion, empyema, necrotizing pneumonia, and lung abscess) and systemic complications (eg, bacteremia, metastatic infection, multiorgan failure, acute respiratory distress syndrome, disseminated intravascular coagulation and, rarely, death). A complication is suspected in the absence of a therapeutic response 48-72 hours after the start of treatment. It is necessary to do a radiograph of the chest. Ultrasound should be performed in all children with empyema, and it is the best technique for distinguishing between pleural fluid and consolidation, assessing the size of the effusion and the degree of complexity, showing the presence of fibrin septations and representing the standard in clinical monitoring of the effect of the applied therapy. Routine computed tomography should not be performed; it is indicated in complicated empyemas and the absence of an adequate therapeutic response or in cases of suspicion of a tumor. Necrotizing pneumonia is extensive proliferation and collicuation of lung tissue despite administration of appropriate antibiotics. Etiology unclear (possible vascular occlusion but also genetic predisposition). A lung abscess is a thick-walled cavity within the lung tissue that contains purulent fluid. It is a consequence of inadequate or delayed treatment of lobar pneumonia. The initial choice of antibiotics is conditioned by the most common causative agents *Streptococcus pneumoniae* and subsequent positive cultures and molecular testing. Moderate to large effusions require drainage and placement of a chest drain. Instillation of intrapleural fibrinolytics such as tissue plasminogen activator (alteplase) and dornase deoxyribonelase alfa through a thoracic drain shortens hospital stay compared with thoracic drainage alone. Open thoracotomy is not recommended as a treatment for empyema in childhood and in recent years has been replaced by video-assisted thoracoscopy (VATS). The advantages over open surgery are that it is minimally invasive and small scars limit tissue damage. Complications of pneumococcal pneumonia treatment involve the intravenous administration of prolonged-course antibiotics.

KEYWORDS: complicated pneumonia; thoracic drainage; fibrinolytics; antibiotics

IL142

THE IMPORTANCE OF VACCINATION AND NATIONAL SURVEILLANCE OF INVASIVE BACTERIAL DISEASES AND WHOOPING COUGH IN SLOVENIA

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In Slovenia, the national laboratory surveillance of invasive diseases caused by *Neisseria meningitidis, Haemophilus influenzae* and *Streptococcus pneumoniae* has been conducted since 1993. Regular vaccination against Hib was introduced at the end of 1999. PCV10 was introduced in 2015 and was replaced by PCV13 in 2019. The whole-cell pertussis vaccine was introduced in Slovenia in 1959 and was replaced in 1999 by acellular vaccine. According to the Infectious Diseases Act, diseases that can be prevented by vaccination must be reported to the National Institute of Public Health. All medical laboratories send invasive isolates of *S. pneumoniae, N. meningitidis* and *H. influenzae* to our reference laboratory for serotyping and/or molecular typing and resistance determination. This project deliberately collects all invasive strains, and the evaluated results reflect the national level. For the characterization of *Bordetella pertussis* in Slovenia and to determine the impact of the change in the type of vaccine, we cultivated 123 isolates during 2002-2020. Vaccine antigen expression was measured, genotyping was performed, and macrolide resistance was tested by PCR. Before the introduction of the Hib conjugate vaccine, this pathogen was an important cause of serious paediatric invasive disease in Slovenia. Due to the introduction of the Hib conjugate vaccine, disease due to invasive *H. influenzae* has become rare. After the introduction of PCV10/PCV13 in Slovenia, a proportional decreases in the incidence and vaccine serotypes among IPD cases in children was observed. Our results highlight the pressure of vaccine selection in pertussis cases. Isolates of *B. pertussis* that do not produce the vaccine antigen PRN appeared after the vaccine was changed, and their prevalence remains high in Slovenia. Continuous national laboratory surveillance of invasive diseases is important for monitoring the effectiveness of the vaccines and vaccination programs. Monitoring of circulating *B. pertussis* isolates is needed to maintain optimal vaccination programs for the prevention of pertussis.

KEYWORDS: vaccine; pertussis; *Haemophilus influenzae; Streptococcus pneumoniae; Neisseria meningitidis*

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PP143

DETERMINATION OF THE FREQUENCY OF HPV INFECTION IN ANORECTAL SWAB SAMPLES OF HIV-POSITIVE MEN

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More than 200 genotypes of human papillomavirus (HPV) are known. HPV infections are common sexually transmitted diseases. Oncogenic high-risk subtypes HPV16 and 18 can cause anal cancer. Men who have sex with men (MSM) population is susceptible to anorectal lesions, and HIV-positive male patients (PLHIV) have a high rate of anal malignancies. The aim of our research was to determine the frequency of HPV infection and the presence of high-risk HPV genotypes in anorectal swab samples of PLHIV men. The study included 87 HIV positive male patients (average age 38.5 ± 8.6 years) treated on an outpatient basis at the UKCS Clinic for Infectious and Tropical Diseases. They had no reported alterations or health issues in the anorectal region and had not undergone testing for the presence of HPV. Testing was done from November 2021 to March 2022. The research was approved by the Ethical Committee and carried out in the Medical Microbiology Service-Virology Laboratory Department of the UKCS. Samples were analyzed on the ELITe InGenius system using the High Risk HPV ELITe Panel test for the detection and differentiation of DNA of 14 high-risk HPV subtypes. Of the 87 analyzed patients, 53 (60.9%) had HPV infection, while 34 (39.1%) were negative. Out of 87 samples, 36 individuals (67.9%) were found to be infected with a single HPV subtype, while 17 participants (32.1%) had concurrent infections with two HPV subtypes. Furthermore, 28.3% of patients tested positive for HPV16 genotype, and 17% were positive for HPV18 genotype. According to previous studies prophylactic HPV vaccination can prevent anal cancer in 95% of HIV-positive homosexual men. Given the prevalence of oncogenic HPV subtypes identified in this study, we advocate for HPV screening among men living with HIV, coupled with proactive HPV vaccination, as a potential strategy to mitigate the incidence of anal cancer.

KEYWORDS: human papilloma virus; HIV-positive male patients; anal malignances; anorectal swab; HPV vaccination

PP144

PERTUSSIS VACCINE OVERVIEW

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Pertussis or whooping cough is a highly contagious, vaccine-preventable, respiratory disease caused by Bordetella pertussis, and transmitted through the respiratory tract. According to the reports of the World Health Organization and Centers for Disease Control and Prevention, the incidence of pertussis shows periodical variations in certain regions of the world. As humans are the sole reservoir of this bacteria complete vaccination against pertussis and high vaccination coverage is of utmost importance for reducing the incidence and severity of the disease. Two types of pertussis vaccine are available: whole-cell (wP) and acellular pertussis vaccine (aP). wP contains whole nonviable bacteria, while aP usually contains two or more protein components. These protein components include inactivated pertussis toxin, filamentous hemagglutinin, pertactin, and fimbriae. The acellular vaccine was developed in response to reports of adverse reactions upon administering the whole-cell vaccine in certain countries. Both vaccines are usually formulated with diphtheria and tetanus toxoids, and more recently a trend of combining more antigenic sources such as Haemophilus influenzae type b, hepatitis B, and inactivated poliovirus vaccine has been accepted in many countries, including Serbia. The wP vaccine stimulates a strong immune response more similar to infection, while the response to aP vaccine differs in this respect. Due to the difference in the types of immune response predominating with different types of pertussis vaccines, there are differences in the duration of protection, and it has been reported that wP induces more durable protection. For countries that have adopted aP increased monitoring is advised as well as the inclusion of booster doses. The special focus is on the vaccination of pregnant women to protect the newborns. Incited by the recent surge in pertussis cases in Serbia here we provide a comprehensive literature overview of pertussis vaccines, covering their historical development, current status, challenges, and potential future directions.

KEYWORDS: whole-cell pertussis vaccine; acellular pertussis vaccine

PP145

CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF LABORATORY-CONFIRMED PERTUSSIS CASES IN VOJVODINA, SERBIA, DURING A SIX-YEAR PERIOD (2018-2023)

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Pertussis is a vaccine-preventable disease and it demonstrates endemo-epidemic pattern every year. Even though its prevalence has decreased with introduction of immunisation, it still remains the important cause of morbidity, especially among children and adolescents. We aimed to determine different characteristics of laboratory-confirmed cases of pertussis among patients whose samples were analysed at the Institute of Public Health of Vojvodina (IPHV), Novi Sad. Nasopharyngeal swab or serum was tested at the Centre for Microbiology, IPHV, using direct (molecular) PCR or indirect (serological) by detection of IgG or IgA. Over the observed six-year period (2018-2023), a total of 3643 patient samples were tested, with 1169 (32.1%) cases of pertussis confirmed. In 2018, 2019, and 2023, 83% of patients were tested positive, while during the COVID-19 pandemic years (2020-2022), only 17% were positive of the total tested samples. The highest number of confirmed cases was in the 10-14 age group (35.2%; 412/1169), both with PCR (43.3%; 101/233) and serological tests (33.2%; 311/936). In the period 2018-2021, the majority of confirmed cases belonged to the ≥20 years' age groups, shifting to the 10-14 age group in the last year of surveillance. The most common referred diagnosis for patients confirmed both by PCR and serological test was "Without first diagnosis" (42.9%), and "Tussis" (31.6%). Under referred diagnosis "Pertussis," only 2.3% (27/1169) of confirmed cases were classified. Pertussis was registered every observed year during a period under surveillance and in all age groups. The shift in age distribution towards older school age supports the introduction of additional pertussis revaccination for children in this age group. By continually educating physicians, the proportion of confirmed pertussis cases among conditions clinically similar to pertussis, would increase, along with the indication for PCR testing within the first two weeks of illness or serological techniques in the following period.

KEYWORDS: Pertussis; laboratory diagnostics of pertussis; PCR technique; serological confirmation of pertussis

PP146

EVALUATION OF KNOWLEDGE AND STIGMA PERCEPTION TOWARDS INDIVIDUALS WITH TUBERCULOSIS IN THE METROPOLITAN CITY OF SKOPJE, NORTH MACEDONIA

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Studies show variations of the level of stigma and variations regarding cultural and geographical reasons for stigmatization of individuals with tuberculosis. Lack of specific knowledge and stigmatization could lead to delay in diagnosis and has negative influence on treatment adherence. This is a cross sectional study conducted in the metropolitan city of Skopje, North Macedonia. A total number of 98 respondents of both genders were included in the study. Evaluation of knowledge and stigma perception towards individuals with tuberculosis was evaluated following anonymously online distributed World Health Organization's Knowledge Attitude Practice (KAP) modified template questionnaire consisted of 21 questions. Descriptive statistics in terms of frequencies or percentages was used for results presentation. Out of 98 respondents, 83% are informed that tuberculosis is an infectious disease. Although 50% of respondents are properly informed for the route of the transmission, namely 50% think that tuberculosis is transmitted through physical touch or use of utensils. High proportion (90%) of respondents properly identified at least one of the tuberculosis symptoms. Considering stigma evaluation, a considerable proportion (35%) prefer not to have any contact with an individual with tuberculosis, except if it is a family member or intimate partner, where this proportion decreases to 20%. There is an important number of respondents (34%) that are concerned that they will be stigmatized if eventually acquire tuberculosis. It is of note that 63% of the respondents would not like their child to play in proximity to an individual with tuberculosis. Our results indicate that our study group, although in general, is fairly informed for tuberculosis and its transmission, an important proportion of respondents is prone to stigmatization of people living with tuberculosis. Therefore, there is an urgent need to overcome those gaps through the implementation of adequate public health strategies.

KEYWORDS: knowledge; stigma; tuberculosis; questionnaire

PP147

DIPHTHERIA AND TETANUS VACCINES: A HISTORICAL OVERVIEW, PRESENT ACHIEVEMENTS, AND FUTURE DIRECTIONS

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Diphtheria and tetanus, once formidable causes of morbidity and mortality worldwide, have seen their threats markedly diminished through the advent and widespread use of vaccines. This review article delves into the historical journey of diphtheria and tetanus vaccines, evaluates their current status in global immunization programs, and explores future perspectives in their evolution and implementation. The inception of diphtheria and tetanus vaccines marked a pivotal shift in infectious disease control. The development of diphtheria toxoid by Emil von Behring in the late 19th century and the subsequent creation of tetanus toxoid in the early 20th century set the stage for large-scale immunization efforts. These efforts were bolstered in the mid-20th century with the integration of these toxoids into combination vaccines, notably the DTP (diphtheria-tetanus-pertussis) vaccine, facilitating broader immunization coverage and enhanced public health outcomes. Currently, the inclusion of diphtheria and tetanus vaccines in national immunization schedules has led to a significant decline in the incidence of these diseases globally. However, challenges remain, including disparities in vaccine coverage and the emergence of non-toxigenic strains causing diphtheria. The review highlights the WHO's strategies towards achieving higher immunization coverage and the importance of maintaining high vaccination rates to prevent resurgence. Looking forward, the review discusses the ongoing research and development aimed at improving vaccine formulations, reducing adverse reactions, and enhancing the efficacy and durability of protection. Innovations such as nanoparticle vaccines and DNA vaccines are explored as potential avenues for future advancements. Additionally, the review addresses the critical role of global health governance in addressing vaccine hesitancy, improving access in low-resource settings, and coordinating responses to outbreaks. In conclusion, while the battle against diphtheria and tetanus has seen significant victories, continuous efforts in vaccine innovation, policy implementation, and global cooperation are essential to sustain these gains and achieve the ultimate goal of global eradication.

KEYWORDS: diphtheria; tetanus; vaccine

PP148

SECREROME ANALYSIS OF B. PERTUSSIS 8/84 VACCINAL STRAIN IN DIFFERENT LIQUID MEDIA

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Bordetella pertussis is an important human pathogen causing serious infections in infants. Acellular pertussis vaccines are composed of purified secreted/surface proteins including inactivated pertussis toxin, as the most important component. This study presents a secretome analysis of one of the four strains included in whole cell pertussis vaccine produced by Institute Torlak. For fermentation, three different media were used; Cohen-Wheeler broth, casein hydrolysate/yeast extract (CH), and casein acid hydrolysate/yeast extract (CAH) based broths. Upon cultivation for 48h, the bacteria were pelleted by centrifugation, prior to 60% ammonium sulfate precipitation. The pellet was dissolved in distilled water and subjected to methanol-chloroform precipitation. Proteomic investigation of the precipitates were done by in-gel trypsin digestion followed by label-free relative quantification on a nano LC-ESI-MS/MS system. Bacterial growth was similar in different growth media, but lower protein levels were detected in Cohen-Wheeler broth. The mass spectrometric analysis of CAH identified 143 proteins. Proteins with Exclusive unique peptide count >10 were: 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, chaperonin GroEL, glutathione-binding protein GsiB, filamentous hemagglutinin, zinc protease, ABC transporter periplasmic amino acid-binding protein, malate synthase G, Tol-Pal system protein TolB, pertactin autotransporter, periplasmic solute-binding protein, aspartate-semialdehyde dehydrogenase, outer membrane porin protein BP0840, adenosylhomocysteinase, malate dehydrogenase, BrkA autotransporter, exported solute binding protein, N-acetyl-gamma-glutamyl-phosphate reductase. Experimental media CAH B. pertussis secretome included two acellular vaccine components, but very low pertussis toxin level. This strain has PtxP1 promoter sequence and it was concluded that this media must be supplemented with heptakis(2,6-di-O-methyl)-*β*-cyclodextrin for toxin purification to be feasible. This is an example of a one page abstract fitting to the needs for publishing in the collection of abstracts. Please do not forget to delete the instructions for writing the abstract.

KEYWORDS: *Bordetella pertussis*, secretome, growth media

MULTI-DRUG AND PAN-DRUG RESISTANCE / HEALTH MICROBIOLOGY AND BIOTECHNOLOGY

IL149

GENOMIC EPIDEMIOLOGY OF CARBAPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA*

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Pseudomonas aeruginosa is an important pathogen causing a range of community and hospital-acquired infections. World Health Organisation designated carbapenem-resistant *P. aeruginosa* (CRPA) as a critical priority pathogen that desperately requires new treatment options. This multicentre study aimed to provide data on antimicrobial susceptibility, underlying resistance mechanisms, genetic context of metallo-β-lactamase (MBL) genes, and clonal relationships between clinical isolates of *P. aeruginosa* carrying MBL genes. State-of-the-art sequencing technologies were used for evaluated genomic characteristics and relatedness of the tested *P. aeruginosa*. Overall, *P. aeruginosa* was detected in 320 out of 5334 (6%) isolates that were collected between 2018 and 2021 from patients admitted to various hospitals throughout Serbia. Whereas CRPA isolates were screened for the presence of the *bla*CTX-M-2, *bla*PER, *bla*TEM, *bla*SHV, *bla*VEB, and *bla*GES, MBL-positive isolates were examined for the existence of the *bla*VIM, *bla*IMP, and *bla*NDM genes. Multilocus sequence typing (MLST) was carried out for *P. aeruginosa*-producing MBL. In addition, four genomes of *P. aeruginosa* harbouring the *bla*NDM gene together with 161 previously published genomes of the same STs, available in the NCBI Pathogen Detection database were subjected to phylogenomic analyses. The majority of the isolated *P. aeruginosa* were recovered from the lower respiratory tract (n=120; 37.5%) and wound specimens (n=108; 33.75%). CRPA isolates accounted for 43.1% (n=138) of the tested isolates, 31 out of them being *bla*NDM-1-positive (22.5%). The prevalence of colistin and ceftazidime-avibactam resistance was 0.3% and 39.5%. MLST analysis revealed the occurrence of ST235 (n=25) and ST654 (n=6), mostly confined to Serbia. The distribution of the beta-lactamase-encoding genes in these isolates suggested clonal dissemination of the following genotypes: ST235/*bla*NDM-1, ST235/*bla*NDM-1/*bla*PER-1, ST654/*bla*NDM-1, ST654/*bla*NDM-1/*bla*PER-1, and ST654/*bla*NDM-1/*bla*GES-5. ST235 and ST654 identified for the first time in Serbia, are high-risk clones and key vectors of acquired MBL and ESBL which is a matter of considerable concern.

KEYWORDS: carbapenem-resistant *Pseudomonas aeruginosa*, metallo-β-lactamase, MLST

ACKNOWLEDGEMENT: Funded by an independent grant from Pfizer.

IL150

FROM SOIL TO LAB: EXPLORING TOXICOLOGY WITH *CAENORHABDITIS ELEGANS*

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Caenorhabditis elegans is a free-living, non-parasitic, fully transparent, bacteriovorous soil nematode. Typically found in temperate climates, it primarily inhabits organic-rich soil and decaying fruit. Nearly six decades ago, Sydney Brenner foresaw its potential as an ideal model system for problems related developmental biology. Over time, *C. elegans* has become instrumental in investigations spanning aging, longevity, host-pathogen interactions, developmental biology, evolution, toxicology and ecotoxicology. With more than 1200 research articles published each year, today *C. elegans* is actively studied in over a thousand laboratories worldwide. Despite its small size, with adult hermaphrodites possessing only 959 somatic cells and 302 neurons, *C. elegans* exhibits a diverse array of specialized tissues, including reproductive, digestive, endocrine, neuromuscular, and sensory systems. Moreover, this nematode shares a remarkable number of conserved genes and signalling pathways with humans, further enhancing its relevance not only in biomedical research but also in toxicology and ecotoxicology. In 1998, *C. elegans* became the first multicellular organism whose genome was completely sequenced. This nematode is an excellent animal model for ecotoxicity assessment because of its translucent body, genetic manipulability, ease of cultivation, rapid and short life cycle that is easily controlled by temperature changes. The assessment endpoints for the toxicology researches are various and include number of live/dead worms, broad size, number of eggs, embryo hatchability, locomotion behaviours, germline apoptosis, oxidative stress and gene expression in *C. elegans*. In our laboratory, *C. elegans* is used in safety and ecotoxicological evaluations of plastic degradation products, artificial and natural materials, as well as antimicrobial substances obtained through the activity of specific microorganisms and their chemical modification in the laboratory.

KEYWORDS: *C. elegans;* toxicology; environmental safety

IL151

ACINETOBACTER BAUMANNII RESISTANT TO LAST-LINE ANTIBIOTICS: AN EMERGING THREAT IN THE WESTERN BALKANS

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Acinetobacter baumannii is considered one of the greatest threats to public health on a global scale. This Gram-negative pathogen causes severe infections, mostly of nosocomial origin, with a high mortality rate. In recent years, the rapid increase in the emergence and spread of antibiotic resistance in *A. baumannii* has significantly limited the effective therapeutic options against infections caused by this bacterium. The last-line antibiotics used in the treatment of multidrug-resistant (MDR) *A. baumannii* are carbapenems, tigecycline and polymyxins. However, resistance to these antibiotics is steadily increasing, especially to carbapenems, leading to an extensively drug-resistant (XDR) and even pandrug-resistant (PDR) phenotype of *A. baumannii*. In 2021, the European Centre for Disease Prevention and Control (ECDC) reported that resistance of *Acinetobacter* spp. to carbapenems reached 50% or more, mostly in Southern and Eastern European countries. Although the Western Balkans is a part of this region, detailed studies on the epidemiology and antibiotic resistance of *A. baumannii* are mainly limited to Serbia and Croatia. In most cases, carbapenem resistance in *A. baumannii* is due to the production of carbapenemases, in particular b-lactamases belonging to the class D known as oxacillinases. The studies from the Western Balkan countries revealed that besides the intrinsic *bla*OXA-51-like gene, the most prevalent acquired oxacillinase gene was the *bla*OXA-24-like followed by the *bla*OXA-23-like, while the *bla*OXA-58-like and metallo- b-lactamase *bla*NDM-1 genes were less common. Although significantly lower compared to carbapenem-resistant, the number of *A. baumannii* isolates resistant to tigecycline and colistin is on a continual rise in the Western Balkans. As worldwide, the main mechanism conferring tigecycline resistance to *A. baumannii* from the Western Balkans was overexpression of efflux pumps. Also, the majority of reported alternations leading to colistin resistance in *A. baumannii* were found in the *pmrCAB* operon, which is responsible for the modification of the colistin target, LPS.

KEYWORDS: *Acinetobacter baumannii*; antibiotic resistance; carbapenems; tigecycline; colistin

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IL152

CHALLENGES AND LESSONS LEARNED FROM ANTIMICROBIAL RESEARCH PROJECTS

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With the surge of antimicrobial resistance, many drugs routinely used in clinical practice are failing to treat infectious diseases. Tackling this antimicrobial resistance emergency requires a multifaceted approach, including the discovery and development of new antimicrobial agents. During this talk some challenges and lessons learned from antimicrobial research projects will be discussed. In addition to resistance, bacteria can also survive lethal antibiotic treatment by developing antibiotic tolerance, more specifically, antibiotic tolerance through persistence. This phenotypic variation seems omnipresent among bacterial life, is linked to therapy failure, and acts as a catalyst for resistance development. Recently, we have described the first proof of pneumococcal persister formation (Geerts et al., Microbiology spectrum, 2022). We have also characterized a large set of pneumococcal clinical isolates, both phenotypically (growth, virulence, antibiotic susceptibility and persistence) and genotypically (genomic sequence), to determine the clinical relevance of pneumococcal persisters. Vulvovaginal candidiasis (VVC) is a vaginal fungal infection commonly caused by the yeast *Candida albicans*, affecting as many as one in every two women during their life. Up to 20% of VVC patients develop severe to recurrent VVC where reinfection occurs more than four times per year. There is a high unmet need for novel treatment options. In a project between industry and academia, we have demonstrated that the activity of Miconazole when combined with the Miconazole potentiator Domiphen Bromide, can combat *in vitro* and *in vivo* the occurrence and recurrence of mucosal biofilm-related vaginal *Candida* infections. Recently, positive results from phase 2 trials in patients with VVC have been published, showing that academia and industry can be good partners in drug discovery.

KEYWORDS: antimicrobials; persisters; *Streptococcus pneumoniae*, *Mycobacteria*

OF153

HIGH-LEVEL RESISTANCE OF CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE TO NOVEL β-LACTAM-β-LACTAMASE INHIBITOR COMBINATIONS IN CLINICAL SETTINGS IN SERBIA

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Klebsiella pneumoniae is one of the most common nosocomial Gram-negative pathogens with an increasing prevalence of carbapenem resistance. Recently, new beta-lactam/beta-lactamase inhibitor combinations became one of the last therapeutic options for the treatment of CRKP infections. A total of 161 isolates of *K. pneumoniae* recovered from 8 hospitals in Serbia in 2022 were included in the study. All carbapenem-resistant *Klebsiella pneumoniae* (CRKP) were further subjected to antimicrobial susceptibility testing against ceftazidime/avibactam, imipenem/relebactam, and meropenem/vaborbactam using gradient strip test. The susceptibility of other antibiotics was evaluated using the disc diffusion method, except for colistin when micro broth dilution method was used. Genes encoding carbapenemases were detected using multiplex PCR, while sequence types were determined using MLST.Resistance rates to beta-lactam/beta-lactamase inhibitor combinations among the tested strains were high: ceftazidime/avibactam (50%), imipenem/relebactam (58.5%), meropenem/vaborbactam (58.5%). Colistin resistance was observed in 54.9% of CRKP isolates. Overall resistance to other antibiotics was also high: ampicillin/sulbactam (98.8%), aztreonam (97.6%), aminoglycosides (84.1%), fluoroquinolones (100%), trimethoprim/sulfamethoxazole (87.8%). Among colistin-resistant CRKP, ceftazidime/avibactam, imipenem/relebactam, meropenem/vaborbactam resistance were 33.3%, 60%, and 57.7%, respectively. The most common carbapenemase in CRKP isolates was NDM-1 found in 47.6% isolates, while OXA-48 was observed in 46.3% of isolates. Two CRKP isolates had both NDM-1 and OXA-48. KPC-2 was found in 6% of CRKP isolates. The MLST showed that the most prevalent sequence type of CRKP isolates was ST147 (32.9%), followed by ST101 (21.9%), ST437 (21.9%), ST11 (12.2%), ST258 (3.6%), ST307 (2.4%), ST395 (2.4%), ST39 (1.2%). The two most common CRKP clones detected were ST147/NDM-1 (34.1%) and ST101/OXA-48 (19.5%).

KEYWORDS: carbapenem resistance; *Klebsiella pneumoniae*; ceftazidime/avibactam; ST147/NDM-1

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GR154

UREAPLASMA UREALYTICUM AND *MYCOPLASMA HOMINIS* IN WOMEN OF REPRODUCTIVE AGE IN CENTER FOR PUBLIC HEALTH SKOPJE, A RETROSPECTIVE ANALYSIS

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Genital *Ureaplasma urealyticum* (U.u) and *Mycoplasma hominis* (M.h) are only considered pathogenic at a certain level and are often associated with other pathological situations such as bacterial vaginosis (BV). They may lead to infertility as well as other gynaeco-obstetrical and neonatal problems. Cervical secretions were collected from women aged 16-56 years consulting for a cytobacteriological examination of the cervical swab associated with simultaneous search for genital mycoplasma in urogenital laboratory at Center for Public Health Skopje. Simultaniously were tested bacterial vaginosis with cultural and microscope method, while genital mycoplasma identification and antibiotic susceptibility testing were performed using the COMPLEMENT Mycofast revolutioN 2 kit. From a study period from January - December 2023 a total of 5846 patients, 364(6.22%) were positive and 5482(93.8%) negative. From a total 231(3.9%) were positive to U.u titer>10.000, 101(1.72%) with titer>100.000 and 32 (0.55%) M.h with titer>10.000. Antibiotics used for *Ureaplasma urealyticum* were: Levofloxacin, Moxifloxacin, Erythomycin, Tetracycline, Doxycycline; as for *Mycoplasma hominis* were: Doxycycline, Levofloxacin, Moxifloxacin, Clindamycin, Tetracycline. Resistance showed in only 25 patients (0,42%) on Tetracycline and Doxycycline. The prevalence of U.u and M.h genital infections is very high in women with bacterial vaginosis. The treatmant of these infections is with a group of antibiotics: Macrolides, Tetracyclines and Fluoroquinolones. These patients showed on both groups resistance on Tetracycline and Doxycyline. Antibiotics such as Fluoroquinolones and Macrolides should be taken in consideration as a therapy of first line for treatment of these infections.

KEYWORDS: BV; *Mycoplasma hominis*; *Ureaplasma urealyticum*; resistance

GR155

MOLECULAR DETECTION OF SCHISTOSOMIASIS USING REAL-TIME PCR PRE- AND POST-TREATMENT IN SOME REGIONS OF NIGERIA

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Schistosomiasis, a neglected tropical disease, is a major helminth disease in terms of morbidity and mortality which infects over 200 million people worldwide. Novel diagnostics for detection of infections are important considering that the current parasitological techniques, though specific, are not very sensitive and not always able to judge accurately the efficacy of Praziquantel (PZQ) in terms of adult worm death but rather in terms of the cessation of egg excretion. A highly sensitive Real-Time PCR (RT-PCR) used for the detection of *Schistosoma sp.* DNA in both urine and faeces sample was compared with conventional PCR and microscopy detection of schistosome eggs. Both urine and stool samples were collected from study participants aged between 3 and 25 years before and after (3 weeks and 8 weeks) treatment with PZQ in Dumbi community, Nigeria. Utilising the diagnostic methods, microscopy detected *Schistosoma haematobium* eggs in 7.5% (29) urine samples collected before treatment whereas RT-PCR amplified DNA in 39.8% (154) of the same samples and no eggs were detected from stool samples analysed. Comparing the diagnostic methods from 50 specimens that were sampled for comparative analysis, RT-PCR had the highest positive detection of 80%, followed by conventional PCR which had 72%, followed by haematuria which had 64% and microscopy had 58%. RT-PCR and conventional PCR both provided lower estimations of the cure rates as compared to microscopy. Results of this study using statistical and bioinformatics methods showed RT-PCR and conventional PCR to be significantly more sensitive than microscopy in detecting and evaluating infection prevalence, an important aspect of epidemiological studies. Thus, RT-PCR based detection technique can be especially useful in circumstances of lower intensity or prevalence of infection, a condition for which the parasitological diagnosis shows a well-proven limitation of its sensitivity.

KEYWORDS: praziquantel (PZQ); real time PCR (RT-PCR); bioinformatics; *Schistosoma haematobium*

GR156

MICROENCAPSULATED ESSENTIAL OILS FROM TWO LAMIACEAE SPECIES FOR COMBAT AGAINST MULTI-RESISTANT *ACINETOBACTER BAUMANNII*

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Antimicrobial resistance (AMR) threatens to increase its mortality rate to 10 million deaths per year by 2050, and *Acinetobacter baumannii* stands out with its insensitivity to almost all available therapeutic options. The risk factors for the acquisition of *A. baumannii* infections have become more frequent during the coronavirus disease 2019 (COVID-19) pandemic, consequently leading to increased AMR. Potential solutions may be explored among naturally derived products, where essential oils (EOs) stand out with a broad spectrum of antimicrobial activity, complex chemical composition, and non-specific mechanisms of action postponing AMR development. However, their volatile nature may affect stability during shelf-life. Thus, we aimed to develop stable and effective microencapsulated systems of essential oils (EOs) derived from two species from Lamiaceae family, *Origanum heracleoticum* L. (oregano) and *Thymus vulgaris* L. (thyme). Microencapsulated complexes of oregano and thyme EOs (OEOC and TEOC) were prepared with hydroxypropyl-β-cyclodextrin as a carrier, and freeze-dried. Fourier-transform infrared spectroscopy verified the formation of inclusion complexes, while thermal stability was confirmed by differential scanning calorimetry. The microdilution broth assay revealed higher antimicrobial activity of the OEOC sample compared to TEOC against 64 *A. baumannii* isolates recovered from COVID-19 patients admitted to intensive care units (MIC values 0.4–1.6 mg/mL for OEOC, and ≥ 1.76 mg/mL for TEOC). Sub-inhibitory concentrations of microencapsulated EOs significantly decreased the biofilm formation of four *A. baumannii* isolates, representatives of a group of isolates based on the genetic pattern (Isolates 1, 2, 39, and 54). Concerning Isolate 2 (representing 60 of the total 64 isolates), the reduction was achieved for more than 50% by both samples. Contrary to antimicrobial potential, TEOC displayed slightly better antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. These results highlight the potential of microencapsulated oregano and thyme EOs in the treatment of infections caused by *A. baumannii*.

KEYWORDS: *Acinetobacter baumannii*; oregano essential oil; thyme essential oil; microencapsulation; antibiofilm

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UNVEILING THE STORY: A CASE REPORT OF ALPHA-HEMOLYTIC STREPTOCOCCI-INDUCED ENDOCARDITIS

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The most clinically significant alpha-hemolytic streptococci include species like *Streptococcus pneumoniae* and viridans streptococci (VS). Due to their ability to form biofilms, VS are among the most common causes of subacute bacterial endocarditis (SBE), primarily in individuals with pre-existing damaged heart valves, commonly with congenital heart disease (CHD). In our case, a 36-year-old female patient had CHD of univentricular heart, significant pulmonary artery stenosis, and left pulmonary artery hypoplasia. In March 2022, she presented with recurrent fevers over a period of several days, reoccurring after the course of amoxicillin with clavulanic acid. Given her medical history of previous staphylococcal endocarditis, she was admitted due to suspicion of SBE. Laboratory analyses revealed elevated C-reactive protein (CRP), with the level of 12.8 mg/l Echocardiographic evaluation revealed an irregular morphology and thickened tricuspid and aortic valve, but no definitive vegetation. Subsequent blood cultures confirmed the presence of *Streptococcus sanguinis*, an alpha heamolytic streptococci from the viridans group. With the treatment course of rifampicin and meropenem, the SBE was fully resolved. In September 2023, she experienced a recurrence of symptoms, including fever, chills, and fatigue. Concerns for recurrent SBE prompted her readmission to the hospital. Once again, laboratory analyses revealed elevated CRP levels of 7.7 mg/l. Three blood cultures yielded positive results for alpha-hemolytic streptococcus. Echocardiography revealed the presence of subvalvular masses, suggestive of vegetation, leading to a diagnosis of SBE. The antibiogram showed resistance to erythromycin, while it showed sensitivity to other tested antibiotics from the groups of penicillins, cephalosporins, fluoroquinolones, carbapenems, glycopeptides, as well as rifampicin and clindamycin. Dual antibiotic therapy was initiated according to the antibiogram for 28 days. The patient remained afebrile throughout, in good general condition, had laboratory analysis within reference values as blood cultures remained sterile. Therefore, the alpha-hemolytic streptococci must be considered in clinically suspicious SBE cases.

KEYWORDS: alpha-hemolytic streptococci; subacute bacterial endocarditis; antibiotic treatment

PP158

ORAL BIOFILMS AS A SOURCE OF PROBIOTICS

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Oral biofilms of healthy individuals can be regarded as a source of beneficial bacteria, as they produce antimicrobial compounds, prevent the attachment of pathogens or compete with the pathogens for the same niches. When treating disease states, such bacteria can be reapplied back to the oral surfaces in combination with other interventions, like physical removal of biofilms and antibiotic treatment. Since the succession of an oral biofilm is dependent on key species, populating the oral epithelia with these species prevents the co-colonization of pathogens and helps restore the healthy oral microbiota. In several isolation campaigns we have collected a large collection (several hundred) of oral strains from healthy individuals, some of which have shown a biocontrol potential against periodontopathogens like *Aggregatibacter actinomycetemcomitans*, *Fusarium nucleatum* and *Porphyromonas gingivalis*. By comparative genomic approach we determined the potential causes of specific antimicrobial activities against gram-negative bacteria and assessed the safety aspects for these strains for their use in clinical applications. For this last purpose we have developed several entrapment based delivery systems, such as nanofibers and microcapsules, which enable us to combine together different bacterial strains into small communities as well as help them efficiently populate target surfaces. It is important that we develop a pipeline for strain isolation, characterization and application of these strains to the oral microenvironments to treat diseases, such as periodontitis. The idea of this application is to guide the reestablishment of a healthy community by excluding the growth of pathogens. It is important to be able to deliver a versatile artificial community of probiotic strains in large cellular numbers. We see the engineering of such artificial communities as a superior approach to the established procedures when trying to re-establishing a healthy oral microbiome.

KEYWORDS: oral microbiome; cell encapsulation; cell delivery; periodontal disease

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PP159

ELECTROCHEMICALLY SYNTHESIZED BIOCOMPOSITE POLY (VINYL ALCOHOL)/CHITOSAN-BASED COATINGS FOR MEDICAL APPLICATIONS

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Osteoarticular implants are designed to facilitate full recovery of lost function and ensure effective fixation of the implant. However, challenges may arise, resulting in implant failure, primarily attributed to infections at the implantation site and aseptic loosening. Thus, the surface of an implant must be altered to simultaneously offer osteoinductive and antibacterial properties. Synthetic hydroxyapatite (HAP) is frequently employed to modify metallic implant surfaces or serve as a bone filler material. To enhance its biocompatibility, HAP-based polymer composites were developed. Among most popular synthetic polymers, poly(vinyl alcohol) (PVA), is widely used because of its similar structure to the native extracellular matrix, along with chitosan (CS), a natural cationic polysaccharide, that shows biocompatibility, biodegradability and antimicrobial action, and could act as a carrier of antibacterial agents. The antibiotic of choice was Gentamicin (Gent), a water-soluble aminoglycoside, with very potent antibacterial activity for the treatment of wide range of infections, caused by Gram-negative and Gram-positive bacteria. The original approach of the presented research is the single-step electrophoretic deposition (EPD) on Ti plates of thus prepared biocomposite that would allow for on-site release of the drug. Obtained hydroxyapatite/chitosan/poly(vinyl alcohol/gentamicin (HAP/PVA/CS/Gent) biocomposite coating exhibited strong antibacterial effect against *E. coli* and *S. aureus*. Gentamicin release study indicated "burst" release in the first 48 h, with ~ 30% of total gentamicin released from the HAP/PVA/CS/Gent coating which is beneficial for the blockage of biofilm formation, followed by slow and steady release in the later period. Cytotoxic effect of HAP/PVA/CS/Gent coating was not pronounced in investigated MRC-5 and L929 cell lines. Also, in MRC-5 fibroblast cells, alkaline phosphatase levels doubled when in contact with HAP/PVA/CS/Gent, indicating good osteogenic properties. The overall construct on the substrate in such a form would be well-advanced arrangement for future medical device improvement of skeletal implants.

KEYWORDS: antibacterial; gentamicin release; poly(vinyl alcohol); chitosan; electrophoretic deposition

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DEVELOPMENT OF ALGINATE/ACTIVATED-CHARCOAL PLATFORM FOR TOPICAL TREATMENT OF RESISTANT PATHOGENS IN CHRONIC WOUNDS

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The aim of this work was to produce novel composites based on either Ca- or Zn-alginate hydrogels and activated charcoal (AC) particles that would, upon contact with physiological fluids, continuously release at least one bioactive agent directly into the wound area. In addition, AC particles served as carriers of other active substances such as povidone iodine (PVP-I), a powerful antiseptic which was used as a model substance. The developed Ca- and Zn alginate composites with incorporated AC particles impregnated with PVP-I were comprehensively investigated *in vitro* regarding its antimicrobial activity against wide range of wild multi-resistant pathogens (MRSA, *E. coli, P. aeruginosa*, *A. baumannii, P. mirabilis*, *E. faecalis*, *C. albicans*), all isolated from patients' wounds. Also, the composites were characterized regarding its textural parameters, morphology, iodine presence, AC and Zn^{2+} ions release profiles as well as iodine adsorption/desorption from AC particles. The obtained composites have exhibited excellent antimicrobial activity. Precisely, synergistic activity of AC particles and adsorbed iodine was shown to be crucial for antibacterial activity while synergy of AC particles and Zn^{2+} ions showed equally strong antifungal effect. However, Zn^{2+} ions alone proved to be selectors of resistant strains of bacteria which could be of relevance in everyday life, since Zn compounds are widely used in ointments and skin preparations from a very early age. Also, it was shown that PVP-I is firmly adsorbed on AC particles and that its release in the surrounding medium is negligible which is very important in regards of preventing often reported systemic iodine absorption after its prolonged medical usage. This novel platform enables further development of efficient multifunctional wound dressings with sustained release of one or more potent bioactive agents *in situ* for prevention and topical treatment of resistant infections and thus address one of the most significant clinical problems today.

KEYWORDS: antibiotic resistance; zinc ions; povidone iodine; antimicrobial activity; sustained release

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PP161

THE IMPORTANCE MOLECULAR DIAGNOSTICS DETECTION RESISTANT MYCOBACTERIUM TUBERCULOSIS STRAINS

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A total of 1.3 million people died from tuberculosis (TB) in 2022. Multidrug-resistant TB remains a public health crisis and a health security threat. Early detection of resistance to antituberculosis drugs in *Mycobacterium tuberculosis* (*M. tuberculosis*) enables prescription appropriate therapy, success treatment and control of TB. In our work, we compare the ability of detection of rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* between molecular Abbott RealTi*m*e MTB RIF/INH Resistance assay (RT-PCR Abbott m2000sp system) and phenotypic drug susceptibility test(AST) on Jansen-Löwenstin agar(LJ). Susceptibility testing was performed with fresh *Mycobacterium tuberculosis* inoculates within 2–3 weeks growth. Susceptibility results were interpreted at 4-and 6-week incubation, respectively. A reference *Mycobacterium tuberculosis* strain H37Rv (ATCC 27294) was included in each test batch as a control. During 2023, 65 samples, in which the presence of M. tuberculosis was confirmed by molecular testing, were tested for the presence of rifampicin (RIF) and isoniazid (INH) resistance genes. Out of the 65 samples (in which the amount of DNA was sufficient for testing), resistance was not detected in half. In 7 samples (7/65) RIF resistance were detected, in 2 samples (2/65) INH resistance and in 2 samples (2/65) both resistance genes were detected. Of 65 samples that underwent AST, 43 samples were identified sensitive to RIF, and 42 to INH, 19 sensitive to both drugs. One strain (1/65) was resistant to RIF, and in 2 samples each, strains were resistant to INH (2/65) or to both antituberculosis drugs (2/65). In the samples in which the presence of the gene of resistance to INH (2) and to both antituberculosis drugs (2) was determined, resistance was phenotypically confirmed. Molecular-based test showed superior ability to detect rifampicin resistance over phenotypic AST method. Detection mutations is molecular indicator of drug resistance *M. tuberculosis* strain, and that resistance must be confirmed on the JI.

KEYWORDS: *M. tuberculosis* resistance, PCR

PP162

THE PROTECTIVE IMPACT OF AQUEOUS EXTRACT OF GINGER ON OXIDATIVE STRESS AND INFLAMMATION OF THE LUNGS OF MALE RATS EXPOSED TO CIGARETTE SMOKE

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Smoking is one of the most remarkable causes of pathogenesis, inflammation, oxidative stress, and changes in the structure and function of the mammal's lung. This study aimed to estimate the impact of chronic smoke exposure on lung tissue and explore if the extract of ginger will help to relieve the pulmonary injury in rats. Four groups of male rats were employed to achieve this goal. The first group was considered as a control group, while the first one of the rest three groups was fed with ginger extract (250 mg/day), the second group was exposed to cigarette smoke, and last group was fed with ginger (250 mg/day) and synchronously exposed to cigarette smoke. Eighteen cigarettes were ignited twice per day for four months. After that, the TNF-α and α -FP levels and parameters of inflammatory cytokines, oxidative stress, antioxidants, and lung histopathology were analysed. The results showed that TNF-α, FP-α, IL-1β and IL-2 levels increased significantly ($p<0.05$) in the serum of rats that exposed to cigarette smoke, while the anti-inflammatory cytokine (IL-22) levels decreased significantly ($p<0.05$). Furthermore, Myeloperoxidase, xanthine oxidase, and malondialdehydes were increased significantly ($p<0.05$) in the lung tissue of rats exposed to smoke, and they decreased significantly in the last group. Antioxidants such as the activity of superoxide dismutase, Glutathione, and total thiol were significantly decreased ($p<0.05$) in the lung tissue of rats exposed to cigarette smoking, while they were significantly elevated in ginger-treated groups. The lungs of rats exposed to smoke exhibited severe congestion as well as an inflammation of these lungs' tissues was observed. The results suggested that the aqueous extract of ginger has protective influences against adverse smoking effects, possibly via the anti-inflammatory and antioxidant of ginger, leading to modulation of oxidative stress and improving the antioxidant defence system. It reduces the pathological alters in rats against lung damage due to smoking.

KEYWORDS: aqueous extract of ginger; antioxidant defence; cigarette smoking; lung; oxidative stress

PP163

BACILLUS CEREUS – NEONATAL SEPSIS CASE REPORT

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Bacillus cereus is a motile aerobic or facultatively anaerobic, Gram positive or Gram variable, spore-forming rods from the family *Bacillaceae*, which is ubiquitous in the environment. *Bacillus cereus* most often causes gastrointestinal disease, but potentially can be serious cause of infection of the bloodstream, lungs and central nervous system of preterm neonates. We report a case of neonatal sepsis caused by *B. cereus* in a premature newborn. Male infant was born at 37th gestational weeks by caesarean section because of reduced quantity and quality of amniotic fluid. Hypotonia and ventral septal defect were observed and child was admitted to the neonatal intensive care unit. The next day tests showed an elevated value of CRP (9,3 mg/l) and the child received double antibiotic therapy parenterally - ampicillin and amikacin. On the 2nd day of life, CRP was increased to 25 mg/l, so blood culture was taken and one more antibiotic (ceftriaxone) was added. Automated system for hemoculture (BacT/ALERT®) signaled positive culture bottle in less than 24h. Gram staining showed spore-forming Gram positive bacilli. Considering that the child still had elevated infection parameters (CRP 15 mg/l), newborn was transported to the Institute of Neonatology with a diagnosis of sepsis. After one day of incubation on 37°C, β-hemolytic, large, yellowish colonies appeared growth on the blood sheep agar. The bacterial isolate was identified as *Bacillus cereus* by an automated system VITEK MS® (MALDI-TOF). The antibiotic susceptibility test showed resistance to erythromycin and susceptibility to imipenem, meropenem, ciprofloxacin, levofloxacin, vancomycin, clindamycin and linezolid, and the neonatologists were immediately informed. The newborn received 10 days of total antibiotic treatment. After complete antimicrobial treatment, additional blood cultures were negative. Prompt and precise diagnosis using automated systems, adequate antimicrobial therapy and good communication between clinicians and microbiologists are crucial for successful outcomes.

KEYWORDS: *Bacillus cereus*; neonatal sepsis; infection

PP164

THE ROLE OF EFFLUX PUMPS IN TIGECYCLINE RESISTANCE OF *ACINETOBACTER BAUMANNII* ISOLATES FROM WESTERN BALKAN HOSPITALS

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The increasing prevalence of multidrug-resistant (MDR) *Acinetobacter baumannii* limits effective therapeutic options, and tigecycline has been considered one of the last resort therapies for MDR *A. baumannii* infections. Nevertheless, *A. baumannii* isolates resistant to tigecycline are becoming increasingly reported, mostly due to overexpression of efflux pumps. The three major RND efflux systems conferring tigecycline resistance in *A. baumannii* are AdeABC, AdeFGH, and AdeIJK, and their expression is regulated by the two-component system AdeRS, the LysR-type regulator AdeL, and the TetR-type regulator AdeN, respectively. Following the above, we aimed to determine the role of efflux pumps in tigecycline resistance of thirty-seven *A. baumannii* isolates collected from Western Balkan healthcare settings (Serbia, Bosnia and Herzegovina and Montenegro) in 2016 and 2022. The majority of isolates belonged to the most prevalent international clonal lineage IC2 (n = 32), four isolates are members of IC1, while only one isolate is identified as IC3. All tested isolates demonstrated a significant decrease in tigecycline MIC in presence of efflux pump inhibitor CCCP (≥16-fold reduction) indicating that mechanism responsible for tigecycline resistance is antibiotic efflux. The comparison of target efflux pump regulatory proteins, translated from nucleotide sequences, to reference strains ATCC19606 and ATCC17978 revealed that most of the isolates have G186V and N268H alternations in AdeS (n = 32), while most common changes in AdeR were V120I and A136V (n = 29) as described in previous studies. Substitution Q262R was detected exclusively in AdeL proteins of IC1 isolates, while no mutations were observed within AdeN regulators. Expression of the *adeB*, *adeG*, and *adeJ* genes in six selected isolates was upregulated in four (1,4- to 3-fold), six (1,6- to 2,6-fold), and three isolates (1,7- to 4-fold), respectively. This study confirmed that overexpression of efflux pump encoding genes enables tigecycline resistance in clinical *A. baumannii* isolates.

KEYWORDS: *Acinetobacter baumannii*; RND efflux pumps; tigecycline resistance; AdeABC; AdeFGH

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EPIDEMIOLOGICAL ANALYSIS OF LYME DISEASE IN THE SOUTH BACKA DISTRICT OF VOJVODINA PROVINCE: A FIVE-YEAR SEROLOGICAL SURVEY EMPHASIZING THE ROLE OF CONFIRMATORY TESTING

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Lyme borreliosis is a multisystem inflammatory infection caused by pathogenic members *Borrelia burgdorferri* sensu lato complex. This vector-borne disease is transmitted to humans by bites of infected ticks from genus *Ixodes*. Signs and symptoms of Lyme borreliosis vary with the stage of the disease and occur as a consequence of interaction between spirochetes and host's immune response. The study objective was to determine the frequency of seroreactivity to *Borrelia burgdorferi* and to gain insight into demographic and geographic characteristics of tested patients in the South Backa District of Vojvodina Province. Additionally, the research emphasizes the importance of confirmatory testing, specially highlighting the role of the immunoblot test to assure accurate diagnosis. The survey covered the results of serological testing on Lyme borreliosis conducted at the Institute for Public Health of Vojvodina for 5 years period (i.e.2017-2022). Patients were tested with both ELISA and Western blot tests according to recommended two-tier procedure. Out of 8916 patients subjected to ELISA testing, 950 exhibited positive results. Among them, 401 were males and 549 females. Western blot testing included 2749 patients, of dose, 116 yielded positive finding, with 44 being male and 72 female. The majority of seropositive patients, as determined by both ELISA and Western blot tests, fell within the age group of 50-70 years. The occurrence of the disease was registered across numerous municipalities in Vojvodina, with significant differences in the number of seroreactivity. No statistically significant differences were observed between genders in any examined year. Among the patients who underwent two-tier testing, Western blot shows less frequent seroreactivity compared to ELISA, suggesting the necessity for confirmation analysis. Our findings confirm the significance of a two-tiered testing approach in ruling out cross reactions among IgM positive patients. They also emphasize the importance of conducting tests only when clinical evidence suggests the diagnosis.

KEYWORDS: confirmatory testing; ELISA; seroreactivity; vector born disease; western blot

PP166

EVALUATION OF ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS AUREUS* AND *ENTEROCOCCUS* SPP. ISOLATES FROM POSITIVE BLOOD CULTURES

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Antimicrobial resistance (AMR) in recent years continuously represents a public health priority. Important causes of nosocomial blood stream infections (BSI) are *Staphylococcus aureus, Enterococcus faecalis* and *Enterococcus faecium* spp. and an Increase of their AMR levels among BSI have been observed in various studies. Our study objective was to investigate the frequency of AMR among isolates of BSI to various antibiotics (cefoxitin, ciprofloxacin, rifampicin, ampicillin, gentamicin and vancomycin). Blood culture bottles (for aerobic and anaerobic cultivation), after inoculation of the inpatients' blood samples, were further incubated in an automated blood culture system (BACT/ALERT® 3D, bioMérieux). When positive result was registered primary bottles were subcultured on standard bacteriological media (Columbia agar, Sabouraud agar and Chocolate agar) for further identification and antimicrobial susceptibility testing (AST). Automated system (VITEK® 2 COMPACT system, bioMérieux) was used for bacterial identification, while for AST both automated and Kirby-Bauer methods were performed. AMR was evaluated according to the criteria defined by the European Committee on Antimicrobial Susceptibility Testing. Out of 165 positive blood cultures, 28%, 12% and 12% were identified as *S. aureus*, *E. faecalis* and *E. faecium*. High percentage (50%) of *Staphylococcus aureus* isolates were found to be methicillin resistant (MRSA), while 21% and 3% were resistant to ciprofloxacin and rifampicin, respectively. Evidently, *E. faecium* was more resistant than *E. faecalis* to all of the tested antibiotics. Namely, resistance to ampicillin, gentamicin and vancomycin was found in 86%, 83% and 76% of the *E. faecium* isolates. However, it is of note that there is also a high frequency of gentamicin resistance in *E. faecalis of* 31%. Relevance of AST among patients with BSI remains an important issue to be addressed to clinical doctors. The prominent level of resistance among blood culture isolates suggests the importance of AST in patients with BSI for providing patients with adequate treatment.

KEYWORDS: blood stream infections; antimicrobial resistance

PP167

PREVALENCE OF *STREPTOCOCCUS PNEUMONIAE* SEROTYPES AND ANTIMICROBIAL RESISTANCE CAUSING NON-INVASIVE DISEASE IN SERBIA

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Streptococcus pneumoniae is an oportunistic bacteria that causes infections, that are non-invasive (acute otitis media, sinusitis, and nonbacteremic pneumonia) and invasive (bacteremic pneumonia, meningitis, and sepsis). It is well known that the introduction of pneumococcal conjugate vaccine (PCV) into the national immunisation program heavily affects serotype distribution and also reduces antibiotic resistance. The study aims to examine serotype distribution and antimicrobial susceptibility of non-invasive *Streptococcus pneumoniae* isolates in Serbia. Non-invasive isolates of *S. pneumoiae* were collected in National Reference Laboratory for streptococci, Institute of Microbiology, Medical Faculty Belgrade from January to December of 2022. Typing of isolates was performed using multiplex PCR method. Susceptibility to antibiotics was tested using disc-diffusion method of antibiogram by EUCAST standard. A total of 155 non-invasive *S. pneumoniae* isolates were enrolled in this study. The representation of patients by age was as follows: ≤ 1 year, 26 (16.1%); >1– ≤ 5 years, 76 (49%); >5–≤18 years, 35 (22.6%); and >18 years, 15 (11.6%). Most strains were isolated from middle ear fluid (N=110, 70.9%). Most frequent serotypes were: 3 (N=25, 16.1%), 11A (N=19, 12.3%), 15A (n=15, 9.7%), 19F (N=12, 7.7%) and 10A (N=11, 7.1%). Coverage of serotypes by PCV10, PCV13 and PCV15 vaccines was 23.9%, 48.4% and 49,7% respectively. The most frequent resistance was to erythromycin (N=43, 27,7%), and non-susceptibility to penicillin was detected in 18.1% of isolates (R=2, 1.3% and I=26, 16.8%). Tetracycline resistance was detected in 27.1% (N=42) of isolates. Constitutive macrolide resistance was the most common (90.7%). The rate of multidrug resistance isolates was 25.2%. Serotype 19F was most resistant compared to other isolates (75% of isolates were resistant to erythromycin and clindamycin, 41.6% were penicillin non-susceptible). A decrease in PCV serotypes and antimicrobial resistance were observed in this study and continued surveillance on pneumococcal isolates may be necessary.

KEYWORDS: *Streptococcus pneumoniae*; antimicrobial resistance; serotype distribution; PCV

ACKNOWLEDGEMENT: Funded by an independent grant from Pfizer.

PP168

ACHROMOBACTER XYLOSOXIDANS - AN UNDERESTIMATED PATHOGEN - THE IMPORTANCE OF ACCURATE DIAGNOSIS AND TREATMENT

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Achromobacter xylosoxidans is an asaccharolytic, non-fermenting Gram-negative, peritrichously flagellated bacillus usually found in soil and water (solutions for disinfection, swimming pools), but also as a part of gastrointestinal and ear microbiota causing infections of various presentations. Phylogenetically, biochemically and according to 16S rRNA sequencing Achromobacter is closely related to the genus Alcaligenes and often morphological misinterpreted for a strain of *Pseudomonas spp.* or *Burkholderia cepacia complex*. During 2023 we isolated *A. xylosoxydans* in seven samples of patients with purulent otitis media and chronical wound infections. We present the case of a 23-years old male with a six months history of right-sided otorrhea, otalgia and subjectively reduced hearing. He had six ENT-specialist visits and diagnosed as cerumen impaction and otitis media. He was treated with ear irrigations and topical fluoroquinolone or aminoglycosides, based on prior laboratory isolation of *Pseudomonas spp.* and antibiotic susceptibility testing. However, the otorrhea and discomfort were not relieved. On ENT exam in October 2023, he was found to have an edematous right external ear canal with a purulent discharge which was sent to our laboratory for analysis. Tested sample was plated on 5% blood sheep agar and chrome agar and after 24h of incubation on 37°C under aerophilic conditions showed growth of oxidase positive and catalase positive colonies which were finally identified by the VITEK MS® (MALDI-TOF) as *A. xylosoxidans*. The antibiotic susceptibility tests for piperacillin-tazobactam, meropenem and trimethoprim-sulfamethoxazole were done according EUCAST. Therapy was switched into trimethoprim-sulfamethoxazole. Patient was completely cured after 10-days of treatment. *A. xylosoxidans* is an emerging, underestimated and biofilm-forming pathogen whose incorrect identification and multiantibiotic resistance (due to numerous constitutive and acquired mechanisms of resistance, especially to fluoroquinolones) can lead to long-term and inadequate treatment. Awareness of its unique susceptibility pattern will lead choosing of appropriate antibiotic therapy.

KEYWORDS: *Achromobacter xylosoxidans*; resistance; ear infection

PP169

THE SIGNIFICANCE OF MOLECULAR DIAGNOSTIC IN ADVANCED HIV INFECTION – CLINICAL CASE

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Antimicrobial resistance is one of the most serious global public health threats. A significant therapeutic problem is represented by multiresistant strains in the hospital, as well as in the outpatient environment. One of the drugs of the last line of therapy - colistin (polymyxin E), binds to lipopolysaccharides and phospholipids in the outer membrane of gram-negative bacteria. Unfortunately, due to the widespread use of colistin in clinical settings and in veterinary medicine, acquired resistance to colistin has also occurred. In addition to chromosomally mediated resistance, a plasmid-mediated colistin resistance gene – mcr (mobile colistin resistance), was also discovered. In a 30-year-old female patient in life-threatening condition who was admitted to the University Clinical Center of Serbia from another health institution, during hospitalization, from: blood cultures, pleural puncture, paratracheal swab, sternum swab, bronchial aspirate and urine culture was isolated Klebsiella pneumoniae subspecies pneumoniae, resistant to all tested antimicrobial drugs (except for tigecycline IE=0.5µg/mL). The production of OXA-48 and NDM carbapenemases was proven by phenotypic methods. *Klebsiella pneumoniae* genes and resistance genes: *bla*SHV, *bla*CTX, NDM, sul1, sul2, aac (6´)-Ib, armA, oqxA, oqxB, OXA48 like and mcr 2, were detected on the HybriSpot device from strains isolated from pleural punctate. In *Enterococcus faecium* sensitive only to high-dose Streptomycin and Quinpristin-Dalfopristin isolated from several blood culture samples, mecA and VanA resistance genes detected (HybriSpot). Upon admission, the patient was treated with Zavicefta, Colistin, Tygacil, Fluconal and Zenix. By applying molecular methods, rapid identification of bacteria, detection of resistance genes, empiric antimicrobial therapy and the application of anti-epidemic measures to prevent the spread of multiresistant strains are enabled, a few hours after the isolation of resistant microorganisms from samples. This is the first case of mcr 2 gene detection in our institution.

KEYWORDS: resistance genes; *mcr 2*; *Klebsiella pneumoniae*; colistin

PP170

ANTIMICROBIAL STEWARDSHIP: AN EXAMPLE OF GOOD PRACTICE AND A MULTIDISCIPLINARY APPROACH IN THE EMERGENCY CENTER UNIVERSITY CLINICAL CENTER OF SERBIA

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Globally, antimicrobial resistance has emerged as a significant threat. A comprehensive plan is required to combat antimicrobial resistance. There have been national and international efforts to address this global health problem, but much work remains. The Centers for Disease Control and Prevention (CDC) reported in 2019 that 2.8 million infections and 32,000 deaths due to resistant pathogens occur each year. Additionally, the World Health Organization (WHO) has endorsed guidance to monitor and evaluate rising antimicrobial resistance. The European Center for Disease Prevention and Control (ECDC) estimates that almost 9 million nosocomial infections (or healthcare-associated infections) occur each year in European hospitals alone, with 1/3 1/3 of these infections being caused by bacteria with some level of AMR. The aim of the paper is to point out the importance of a multidisciplinary approach in the fight against antimicrobial resistance in the Emergency Center of the University Clinical Center of Serbia. World Health Organization Antimicrobial Resistance Division https://www.who.int/antimicrobial-resistance/en/; CDC Training on Antibiotic Stewardship; Antimicrobial stewardship interventions: a practical guide (WHO Regional Office for Europe,2021). The team for the control of the use of antibiotics (AMS team) in UC UCCS, which conducts AMS, was formed in 2015 at the Emergency Center. AMS team consists of an infectious disease specialist, anesthesiologist, clinical physician, / pharmacologist, a microbiologist and an epidemiologist. The team meets once a week and reviews patients registered for consultation and makes recommendations for antibiotic therapy. The importance of teamwork is seen in the AMS, because only in this way can the correct use of antibiotics be influenced, as well as the education of young doctors in this important field. A multidisciplinary approach to solving the problem of antimicrobial resistance is an important way to fight this major public health problem in the world. Prevention of antimicrobial resistance depends on appropriate clinical practices that should be incorporated into all routine patient care. Preventing infections will reduce the burden of MDROs in healthcare settings.

KEYWORDS: antimicrobial resistance; antimicrobial stewardship programs; multidisciplinary approach in antimicrobial stewardship programs

PANEL SESSION - INFECTIONS IN PATIENTS ON IMMUNOMODULATION AND IMUNOSUPRESIVE THERAPIES

PA171

BIOLOGICAL THERAPY AND INFECTIONS IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

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A 6 y.o. boy was first diagnosed with acute lymphoblastic leukemia (common B) in April 2021. He had hyperdiploidy, but no molecular rearrangements and no CNS involvement. He was treated according to ALL IC-BFM 2009 protocol and had good initial therapeutic response, but due to positive MRD D+15 he was stratified in IR group. CR was achieved on D+33 and maintenance therapy initiated. In September 2022 he was diagnosed with 1st very early, isolated, medullary relapse of ALL. ALL-IC REL protocol was initiated but had positive MRD D+29. HSCT from matched, unrelated donor, as the only viable option was performed in January 2024. Although initially HSCT was successful he was diagnosed with 2nd relapse in April 2024 having 60% of blast cells infiltration of BM. Treatment was escalated with biological therapy. He received blinatumomab but mid-treatment developed high grade fever with increased CRP. Broad spectrum antibiotics were initiated and in several days his fever normalized and he was in good clinical condition. Although no isolates were found his prompt and complete response to antibiotic course indicates infectious nature of fever. Hematopoietic stem cell transplant (HSCT) is an important therapeutic option for many malignant and non-malignant ailments. It utilizes high dose chemotherapy, total body irradiation but also biological therapy – either as part of consolidation treatment prior to HSCT, conditioning regimen of HSCT or post HSCT therapy, aimed at graft reactivity control, adjuvant therapy of primary disease or treatment of HSCT related complications. Most commonly used biological drugs are monoclonal antibodies (e.g. anti-thymocyte globulin, rituximab) and in our case blinatumomab, a bispecific antibody prior to second HSCT. Although invaluable therapeutic option biological drugs also have many adverse effects, many of which reduce the ability of immune system to respond to infection. It is sometimes difficult to differentiate between drug related adverse effects and infections, fever being major sign in both scenarios. Fast, precise and correct differential diagnosis significantly improves outcome, while delayed treatment or misdiagnosis can lead to severe consequences and reduce treatment effectiveness.

KEYWORDS: hematopoietic stem cell transplant; biological therapy; HSCT; blinatumomab

PA172

TUBERCULOSIS INFECTION IN THE CONTEXT OF INFLAMMATORY BOWEL DISEASE: A CHALLENGE IN BIOLOGIC THERAPY

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Inflammatory bowel diseases (IBD), such as ulcerative colitis (UC), present significant therapeutic challenges, especially when complicated by infections like tuberculosis (TB). This case report presents management of a patient diagnosed with chronically active extensive UC in 2008, with complications of TB infection during the administration of biologic therapy. The patient was initially managed with mesalamines, however due to frequent relapses in 2012 he was started on Azathioprine which was short-lived due to hepatotoxicity of the drug. The patient than started a clinical trial for a Janus kinase (JAK) inhibitor biologic therapy in 2014, with the therapy being discontinued in November 2015 following the development of fever and ascites. The chest X ray and a CT scan were negative, but a positive Quantiferon Gold test and ascites results were indicative of an active TB infection of unknown location. This situation underscores the increased risk of TB reactivation associated with biologic therapies, particularly JAK inhibitors and anti-TNF agents. Following the reactivation of TB, the patient was treated with antitubercular drugs under pneumologist supervision, which highlights the need for TB screening protocols before initiating biologic treatments in IBD patients. Subsequent treatments included other biologics, finally culminating in Vedolizumab therapy in February 2022, which was chosen for its gut-selective mechanism, considering the patient's previous TB history. However, in 2023 the patient is presenting with cough, low grade fever, weight loss, and an enlarging pulmonary nodule, which was followed by extensive diagnostic evaluation and frequent follow ups. This case illustrates the multifaceted challenges in treating IBD patients with a history of TB, emphasizing the importance of thorough infectious disease screening and tailored therapeutic strategies. It also reflects on the evolving landscape of biologic therapies and the critical role of monitoring for infections, particularly TB, thereby ensuring safe and effective long-term management of IBD.

KEYWORDS: IBD; tuberculosis; biologic therapy

PA173

OCCURRENCE OF PNEUMONIA IN A PATIENT ON BIOLOGICAL THERAPY DUE TO TRAPS

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Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is an autosomal dominant autoinflammatory disease characterized by prolonged and recurrent episodes of fever, abdominal and/or chest pain, arthralgia, myalgia, and erythematous rash. TRAPS is associated with heterozygous variants in the TNFRSF1A gene, which encodes the tumor necrosis factor receptor. Interleukin-1 (IL-1) inhibitors are considered first-line therapies. The patients receiving these agents have a mild to moderate risk of infection. This case presents the boy with TRAPS who developed pneumonia during treatment with biological therapy. Our patient's recurrent episodes of fever accompanied by abdominal pain and diarrhoea started occurring at the age of ten months and repeated every four months. He was treated with antibiotics on several occasions. Intermittent use of prednisone was advised. The genetic testing had been performed and the missense heterozygous variant in TNFRSF1A gene (c.236C>T, p.Thr79Met) was detected. Considering the mentioned findings, the patient was diagnosed with TRAPS. After standard laboratory test and QuantiFERON test, the treatment with human monoclonal anti-IL-1β antibody was planned. The first dose of canakinumab (Ilaris™) was administered in September 2022. Next month, the boy got fever and started coughing. Chest radiography revealed pneumonia. He had been treated with cephalosporins for ten days and the second dose of canakinumab was also administered. In the further course, he has been given antibiotic prophylaxis. Eventually, the episodes of fever and abdominal pain have become shorter and fewer. It is important to carefully decide on therapy discontinuation in case of infection as abrupt withdrawal can induce a flare of the disease. Canakinumab can be effective in suppressing the disease activity, preventing reactive amyloidosis and the progression of organ damage. More studies are necessary to evaluate its long-term effects on TRAPS and to establish specific prevention strategies for possible infections.

KEYWORDS: tumor necrosis factor receptor-associated periodic syndrome (TRAPS); canakinumab; pneumonia

PA174

A SERIES OF RARE INFECTIOUS COMPLICATIONS IN AN IMMUNOSUPPRESSED PATIENT WITH SLE

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Systemic lupus erythematosus (SLE) is a disease infamous for its wide spectrum of clinical manifestations and possible complications. Severe infections are one such group of complications. They usually occur in the course of SLE treatment forcing the treating physician to make difficult therapeutic choices in an attempt to balance the risk of an uncontrolled infection against that of a SLE flare. We present a case of a thirty-three-year-old female with SLE and autoimmune hemolytic anemia and lymphopenia as its major features. She was stable for roughly four years while on prednisone, an antimalarial, and mycophenolate mofetil. Then, in 2020, while being treated as an inpatient for deep venous thrombosis, she contracted a SARS-Cov-2 infection. She received antivirals, but within a month's time, she developed a lung abscess followed by a brain abscess that, the latter of which had to be surgical removed. Nocardiosis was demonstrated in the cerebral abscess by a pathologist. Mycophenolate mofetil was stopped after brain surgery and she was on prednisone, an antimalarial, antibiotics and immunoglobulins for the next two years. During that time, she had multiple bouts of mastoiditis, otitis media and sinusitis for which she had also been operated on and received multiple antibiotics. Finally, she developed fusariosis of the lung for which she received systemic antifungals and was stabilized. However, she then experienced a SLE flare presenting with severe hemolytic anemia that was only resolved after plasma exchange therapy and methylprednisolone pulses. Patients with SLE are prone to life-threatening infection due to longstanding immunosuppressive therapy. The goal is to balance immunosuppression and the risk of infection while remaining vigilant for flares of SLE and signs of infection. The collaboration between different specialists is necessary to traverse such treacherous waters.

KEYWORDS: nocardia infections; lupus erythematosus; systemic

PANEL SESSION - INTRAHOSPITAL AND EMERGING INFECTIONS

PA175

A CASE REPORT OF STAPHYLOCOCCUS AUREUS PROSTHETIC VALVE ENDOCARDITIS IN PATIENT WITH RANDU – OSLER – WEBER SYNDROME

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Endocarditis is a life-threatening condition with a wide spectrum of clinical manifestations. It has high morbidity and mortality, and represents a great diagnostic and therapeutic challenge. It can occur in an acute, subacute and chronic course. The main causative agents are *Staphylococcus* and *Streptococcus*, both native and artificial valves. Persistent bacteremia, during the period of epithelialization of the artificial valve, is the main pathogenetic mechanism of early prosthetic endocarditis. We will present the case of a 61-year-old patient with Randu-Osler-Weber syndrome, who developed endocarditis several weeks after aortic valve surgery and replacement with a biological prosthesis. The main risk factor for persistent bacteremia, was nasal colonisation. Our patient with hereditary hemorrhagic telangiectasia had a high operative risk, but operative treatment was also inevitable. A special therapeutic challenge is lifelong suppressive antistaphylococcal therapy with further hematological evaluation due to recurrent epistaxis and a high risk of relapse.

KEYWORDS: endocarditis; *Staphylococcus*; Randu-Osler-Weber syndrome

PA176

FULMINANT NECROTIZING FASCIITIS: A FATAL OUTCOME. CASE SERIES

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Necrotizing fasciitis (NF) is a rare but potentially fatal infection categorized into three types: type I (polymicrobial), type II (monomicrobial, typically caused by group *A. streptococci* or *Staphylococcus aureus*), and type III (attributed to Vibrio or Aeromonas species). Monomicrobial NF caused by *Escherichia coli* or *Klebsiella pneumoniae*, also classified as type III NF, carries a higher mortality risk. *Klebsiella pneumoniae* NF is rare outside Asia. This case series presents seven patients diagnosed with *Klebsiella pneumoniae* NF in June 2015 at our hospital. Postoperatively, patients developed fulminant NF, sepsis, and in some cases, septic shock, leading to disseminated intravascular coagulopathy, acute renal failure, and leukopenia. Among them, three patients had fatal outcomes. All patients were treated according to the current protocols for the treatment of sepsis and necrotizing fasciitis. *Klebsiella pneumoniae* showing intermediate susceptibility to Meropenem and Imipenem was isolated from blood cultures, wound swabs, or skin samples in all cases. Phylogenetic analysis showed strains of the same origin. Epidemiological surveillance showed the contamination of Propofol used in the operating room with this bacterium. Despite commonly affecting immunocompromised individuals, this series reveals a diverse patient population in terms of age, type of surgery and comorbidities. Lack of travel history to Asia suggests nosocomial infection. *Klebsiella pneumoniae* is increasingly recognized as a significant cause of monomicrobial NF globally, characterized by rapid progression, multifocal spread, and high mortality. In order to prevent the emergence of new bacterial strains, measures should be taken to control the spread of this aggressive bacterium and to use antibiotics rationally. Increased awareness and early intervention, including antibiotic therapy and debridement, are crucial for improving survival rates. Further research is needed to better understand and manage this life-threatening infection

KEYWORDS: necrotizing fasciitis; *Klebsiella pneumoniae*; nosocomial infection

PA177

ASPERGILLOSIS - DIAGNOSTIC AND THERAPEUTIC CHALLENGES, A CASE SERIES

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Aspergillosis comprises a range of infections caused by the *Aspergillus* species, presenting significant diagnostic and therapeutic challenges, particularly for immunocompromised individuals. The complexity of aspergillosis diagnosis and treatment is accentuated by the emergence of antifungal resistance and diverse clinical manifestations. Among the over 180 *Aspergillus* species identified, *A. fumigatus*, *A. flavus, A. niger*, *A. terreus*, and *A. nidulans* are most commonly pathogenic to humans. The prevalence of aspergillosis is on the rise, with invasive aspergillosis becoming increasingly common in patients with compromised immune systems. A variety of diagnostic techniques—including radiological imaging, microbiological cultures, molecular diagnostics, and serological tests for antigens and antibodies—are employed to diagnose the infection. Although real-time PCR and antigen detection are highly sensitive diagnostic methods, approximately 40% of aspergillosis cases remain undetected until post-mortem examinations, underscoring the necessity for improved early detection. Treatment approaches for aspergillosis require a multifaceted strategy. Optimization of existing antifungal therapies is essential, and the development of combined therapeutic regimens is underway to combat resistant strains. Immunotherapy, particularly the use of monoclonal antibodies and immune response modulators, shows potential as an innovative treatment modality. Moreover, preventive strategies are essential, with the implementation of environmental controls such as HEPA filtration systems. In addition to a comprehensive review of aspergillosis, this paper will present a case report that exemplify the condition resulting from immunosuppressive scenario. These cases underline the urgent need for more refined diagnostic techniques and the development of new therapeutic interventions to effectively manage aspergillosis, a disease with a significant impact on public health.

KEYWORDS: aspergillosis; immunocompromised individuals; early detection; invasive aspergillosis; antifungal therapies

PA178

UNRAVELING THE MYSTERY: A CASE OF SEPTIC SHOCK WITH UNKNOWN ORIGIN

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In critically ill patients admitted to the intensive care unit (ICU), sepsis remains a significant challenge, particularly when the source of infection is elusive. Sepsis of unknown origin presents a diagnostic dilemma, often requiring a precise evaluation to identify the causative pathogen and guide appropriate treatment. We present a case of 71 years old female who was brought to our hospital by emergency ambulance, with suspicion of cardiogenic pulmonary oedema. Since she was unconscious, hypotensive, tachycardic and hypoxic she was urgently admitted to the Coronary Unit were invasive mechanical ventilation and vasopressor stimulation were started. FOCUS echo excluded pulmonary embolism and left heart failure as a reason of hemodynamic instability, while laboratory findings were suggestive of multiorgan failure and sepsis. Due to community acquired infection and sepsis she was transferred to our ICU. We collected microbiological samples and continued with dual antibiotic therapy and supportive strategies for treating critically ill patients. Out of all samples tracheal aspirate was positive with *Candida* species which was not treated initially since she did not have any risk factor for invasive fungal infection. However, as she remained hemodynamically instable and limited for further radiological diagnostic, surrogate markers were obtained (galactomannan, beta-d-glucan). On the same day beta-d-glucan was positive but the interpretation of result was limited as she received beta lactam antibiotic for suspected ventilator associated pneumonia. Given the fact that we did not achieve any clinical improvement we decided to introduce echinocandin in therapy. After 48h of starting antifungal therapy clinical improvement was obtained. ICU stay was complicated by drug induced liver damage which eventually resolved. Fifteen days after admission she was extubated and transferred to ward. Although uncommon, candida infection can occur in immunocompetent patients, highlighting the importance of considering fungal pathogens in the differential diagnosis, even in individuals with intact immune function.

KEYWORDS: sepsis; intensive care unit; critically ill patient; *Candida* spp.

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U Pfizer-u smo konstantno u neumornoj potrazi za naučnim otkrićima i revolucionarnim lekovima koji će stvoriti zdraviji svet za sve.[1] Ponosno sarađujemo sa hiljadama studijskih centara i desetinama hiljada ispitanika širom sveta, što omogućava razvoj lekova koji menjaju živote.[2] Sve odluke koje donosimo, usmerene su na očuvanje zdravlja i kvaliteta života naših pacijenata. Našu svrhu ispunjavamo tako što nastojimo da budemo izvor najnaprednije nauke na svetu u saradnji sa partnerima iz sistema zdravstvene zaštite, kako bismo poboljšali pristup Pfizerovim lekovima. Uostalom, jedan od naših glavnih ciljeva je da negujemo okruženje u kojem značajna otkrića mogu napredovati.[3]

Literatura: 1. Pfizer: One of the world's premier biopharmaceutical companies. Dostupno na: https://www.pfizer.com/. Poslednji pristup: 21.02.2023. **2.** Our Science: Pharmaceutical Development. Dostupno na: https://www.pfizer.com/science. Poslednji pristup: 21.02.2023. **3.** Breakthroughs That Change Patients' Lives. Dostupno na: https://www.pfizer.com.hk/en/our-purpose-en/breakthroughs-that-change-patients-lives. Poslednji pristup: 21.02.2023.

Pfizer

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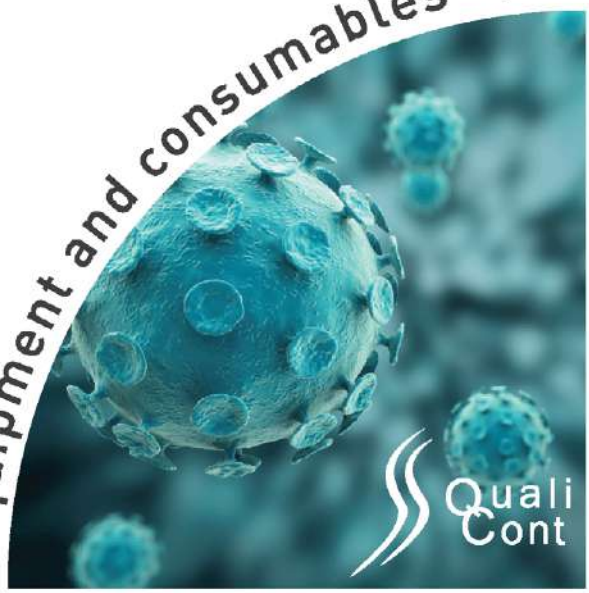

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