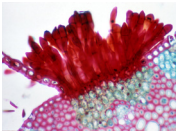
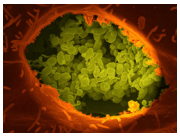
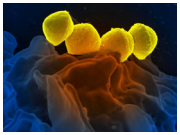
POWER OF MICROBES
IN INDUSTRY AND ENVIRONMENT 2023

Book of Abstracts







May 15-18, 2023 | Valamar Diamant Hotel, Poreč, Croatia

Power of Microbes
in Industry and Environment

BOOK OF ABSTRACTS

May 15 – 18, 2023
Poreč, Croatia

Publisher

Croatian Microbiological Society

Pierottijeva 6, HR-10000 Zagreb, Croatia

www.hmd-cms.hr

For publisher

Roberto Antolović

Editors

Renata Teparić

Andreja Leboš Pavunc

Domagoj Kifer

ISBN 978-953-7778-19-4

Organised and hosted by

Croatian Microbiological Society

Co-organised by

Czechoslovak Society for Microbiology

Hungarian Society for Microbiology

Slovenian Microbiological Society

Turkish Society of Microbiology

Supported by

Croatian academy of Engineering

Supported by

Faculty of Food Technology and Biotechnology, University of Zagreb

Federation of European Microbiological Societies (FEMS)

Gold sponsor

Labena

Silver sponsors

Avantor

Diagnostica Skalpeli

Kemolab

Silver sponsors

Medic

Vita Lab Nova

Bronze sponsors

AlphaChrom

A&B

Gorea plus

Jasika

Selvita

Organising Committee

Renata Teparić, president

Andreja Leboš Pavunc, secretary

Marina Svetec Miklenić, treasurer

Domagoj Kifer

Mirna Mrkonjić Fuka

Ines Sviličić Petrić

Bojan Žunar

International Scientific Committee

Vladimir Mrša, president (Croatia)

Anna Maraz (Hungary)

Marjanca Starčič Erjavec (Slovenia)

Michael Sauer (Austria)

Helena Bujdakova (Slovakia)

Branka Vasiljević (Serbia)

CONTENTS

Presidents' foreword 9

General information 10

Programme 11

Invited lectures 17

Oral presentations 33

Poster presentations 57

Tribune lectures 123

Sponsor lecture 131

List of authors 135

PRESIDENTS' FOREWORD

Dear colleagues and friends,

It is our great pleasure to welcome you at the symposium ″Power of Microbes in Industry and Environment 2023″, held in Poreč, Croatia from May 15th to May 18th 2023.

This symposium which covers all important topics of applied microbiology is already the seventh in a row, starting with the meeting in Opatija in 2002 and followed by the symposia in Zadar in 2007, Malinska (island Krk) in 2010, Primošten in 2013, Krk in 2016 and Sv. Marti na Muri in 2019. Like the previous meetings, the one this year is organized by the Croatian Microbiological Society in collaboration with the Czechoslovak Society for Microbiology, Hungarian Society for Microbiology, Slovenian Microbiological Society and Turkish Society of Microbiology. The symposium is held with the support of the Federation of European Microbiological Societies (FEMS), Faculty of Food Technology and Biotechnology, and Croatian academy of Engineering.

The experience of the past meetings motivated our efforts to continue with this series with a clear tendency to strengthen the scientific connections among research groups of neighbouring countries. Following the tradition established by the previous meetings, ″Power of microbes 2023″ will cover hot topics in the fields of applied microbiology and biotechnology, thus creating multidisciplinary background and bringing together scientists from all research environments, including academia, research institutes and industry. We strongly believe that ″Power of microbes 2023″ is an excellent place to exchange and combine scientific ideas among the experts and participants with great possibilities to start the new international collaborations and common scientific projects. In addition to the lectures of the invited speakers, the programme includes presentations of a number of young scientists and PhD students, many of which are supported by FEMS grants. We thank all participants for their scientific involvement that will significantly contribute to the success of ″Power of microbes 2023″.

We hope that you will enjoy the programme of the ″Power of microbes 2023″ and find it stimulating and informative. We also hope that you will enjoy the beauty of Istria county and Croatian hospitality. Last but not least, we wish that the ″Power of microbes 2023″ will continue to be the place to revive the old and form the new friendships.

Renata Teparić

President of the Organising Committee

Vladimir Mrša

President of the International Programme Committee

GENERAL INFORMATION

SYMPOSIUM VENUE

The meeting is held at the congress centre of the Valamar Diamant Hotel 4*, Brulo 1, HR-52440, Poreč, Croatia. Phone: +385 52 400 000.

REGISTRATION OF PARTICIPANTS

Registration desk will be opened on Monday, May 15 from 14:00 to 15:00, as well as on Tuesday, May 16 from 08:30 to 09:00 in front of the Magnolia congress hall, Valamar Diamant Hotel. Daily updates on the symposium sessions and social events will be available at the registration desk.

All participants and accompanying persons are kindly requested to wear their conference badges during the scientific sessions and symposium social events.

LANGUAGE

The official language of the symposium is English.

INTERNET AND E-MAIL ACCESS

To access the internet and e-mail, please ask at the reception desk of the Valamar Diamant Hotel.

OPENING CEREMONY AND SOCIAL EVENTS

The opening ceremony will be held in the congress hall of the Valamar Diamant Hotel on Monday, May 15 from 15:00 to 15:30. The welcome reception with buffet dinner will take place at 20:00 on the terrace of the hotel restaurant.

On Wednesday, May 17 the symposium excursion is scheduled at 15:00. Excursion includes symposium dinner at family run farm Jadruhi, starting at 19:00.

INFORMATION FOR PRESENTERS

Oral presentations will be held in Magnolia congress hall of the Valamar Diamant Hotel. LCD projections are available during all sessions. Please send your PowerPoint presentation to the powerofmicrobes2023@gmail.com.

Posters will be displayed in the congress hall Lavanda on Tuesday, May 16 and should be mounted during the morning. Presenters of the posters are kindly requested to be at their posters and available for discussion on Tuesday, May 16 from 16:20 to 20:00. Posters should be dismounted immediately after poster session.

PROGRAMME

MONDAY, MAY 15, 2023

Arrival of participants and accommodation

14:00 – 15:00 **Registration**

In front of congress hall (Magnolia)

15:00 – 15:30 **Opening ceremony**

Congress hall (Magnolia)

15:30 – 16:40 **Opening Session**

Chaired by: Roberto Antolović, Renata Teparić

Congress hall (Magnolia)

13:30 – 16:05 IL1 **D. Mattanovich** (Austria): CO_2 Capture and Conversion to Base Chemicals by Engineered Autotrophic Yeast

16:05 – 16:40 IL2 **I. Matić** (France): rRNA Operon Multiplicity as a Bacterial Genome Stability Insurance Policy

16:40 – 17:00 Coffee break

Lobby bar

17:00 – 19:10 **Session 1: Microbes in The Laboratory**

Chaired by: Robert Kourist, Branka Vasiljević

Congress hall (Magnolia)

17:00 – 17:35 IL3 **A. Sibirny** (Ukraine): Non-Conventional Yeasts for Biofuels and High-Value Chemicals

17:35 – 18:10 IL4 **B. Vasiljević** (Serbia): Effect of Free Fatty Acids on *Streptomyces*

18:10 – 18:30 OP1 **T. Márquez** (Austria): Secondary Metabolites from Mono- And Co-Cultures of Bacteria Associated with The Freshwater Bryozoan *Cristatella mucedo*

18:30 – 18:50	OP2	**P. Shetty** (Hungary): Impact of Algal-Bacterial Co-Cultivation on Algal Biomass and Biohydrogen Production
18:50 – 19:10	OP3	**M. Miloloža** (Croatia): Comparison of Bioremediation Strategies for The Biodegradation of Microplastics by Bacteria and Yeast
20:00	Welcome Reception	

TUESDAY, MAY 16, 2023

09:00 – 13:00	**Session 2: Microbes in The Factory**	
	Chaired by: Colin Harwood, Diethard Mattanovich	
	Congress hall (Magnolia)	
09:00 – 09:35	IL5	**R. Kourist** (Austria): Enzyme Discovery and Protein Engineering for The Synthesis of New Biobased Polymers
09:35 – 10:10	IL6	**C. Harwood** (United Kingdom): Optimising *Bacillus subtilis* for Industrial Enzyme Production
10:10 – 10:30	OP4	**M. Zugan** (Slovenia): The Potential of New *Paraburkholderia* sp. Isolates for Upcycling Lignin from Invasive Plant Species
10:30 – 10:50	Coffee break	
	Lobby bar	
10:50 – 11:25	IL7	**H. Pichler** (Austria): Yeast Sterol Engineering
11:25 – 12:00	IL8	**N. Čadež** (Slovenia): Genomic Adaptations of *Saccharomyces* Yeasts to Brewing Environments
12:00 – 12:20	OP5	**O. Schneider** (Austria): Genome Mining of *Streptomyces* sp. S4.7 For Meroterpenoids and Prenylated Phenazines
12:20 – 12:40	OP6	**J. Ruchala** (Poland): Yeasts as Biotechnological Producers of Bacterial Antibiotics Roseoflavin and Aminoriboflavin

12:40 – 13:00	OP7 **Q.D. Nguyen** (Hungary): New Approach for Development of Artificial Microbial Consortium for Biological Pretreatment of Lignocellulosic Biomass
13:00 – 15:00	Lunch break (optional)
15:00 – 17:00	**Tribune: Microbes in Cheese and Wine Production (supported by Agrolaguna)** **Chaired by: Mirna Mrkonjić Fuka** **Congress hall (Magnolia)**
15:00 – 15:15	TL1 **N. Mikulec** (Croatia): The Potential of Microencapsulation in Cheese Production
15:15 – 15:30	TL2 **J. Burtscher** (Austria): Prevention of Late Blowing in Cheese
15:30 – 15:45	TL3 **S. Radeka** (Croatia): Influence of Maceration Time and Temperature on Bioactive Compounds and Antioxidant Capacity in Malvazija Istarska and Teran Wines
15:45 – 16:00	TL4 **I. Lukić** (Croatia): Influence of Non-*Saccharomyces* Yeasts on Chemical and Sensory Characteristics of Malvazija Istarska White Wine with The Emphasis on Volatile Esters as Revealed by Comprehensive Two-Dimensional Gas Chromatography
16:00 – 16:20	Coffee break **Lobby bar**
16:20 – 20:00	**Poster Session** **Congress hall (Lavanda)**

WEDNESDAY, MAY 17, 2023

09:00 – 12:00	**Session 3: Microbes in Environment**	
	Chaired by: Maja Rupnik, Nikolina Udiković Kolić	
	Congress hall (Magnolia)	
09:00 – 09:35	IL9	**G. Maróti** (Hungary): Exploitation of Interkingdom Microbial Interactions in Bioenergy Generation
09:35 – 09:55	OP8	**I. Dimkić** (Serbia): Differential Abundance Analysis of "Core" Bacteriobiota During Key Growth Stages of Maize
09:55 – 10:15	OP9	**I. Babić** (Croatia): Assessing the Health of The Marine Environment by Tracking a Complex Network of Microbial Communities in Sediment
10:15 – 10:35	OP10	**A. Puljko** (Croatia): Extended-spectrum β-lactamase- And Carbapenemase-Producing *Enterobacterales* in Hospital Wastewater in Croatia
10:35 – 11:00	Coffee break	
	Lobby bar	
11:00 – 11:20	OP11	**S. Kajić** (Croatia): Phylogenetic Analysis of Indigenous Rhizobial Strains Nodulating Soybean (*Glycine max* L.) and Their Symbiotic Efficiency Under Drought Conditions
11:20 – 11:40	SL1	**L. Markulin** (Labena, Croatia): eDNA Detection: A Powerful Tool for Monitoring Microbes in The Environment
11:40 – 12:00	OP12	**M. Rusková** (Slovakia): Analysis of Microbial Community and Specific Resistance Genes of The Microplastics, Water and Surroundings of Danube River
12:00 – 14:00	Lunch break (optional)	
15:00	Excursion with dinner (optional)	

THURSDAY, MAY 18, 2023

09:00 – 10:50	**Session 4: Microbes in The Human Body**	
	Chaired by: Roberto Antolović, Aleš Lapanje	
	Congress hall (Magnolia)	
09:00 – 09:35	IL10	**S. Friant** (France): Humanization of Yeast *Saccharomyces cerevisiae* to Study Human Proteins and Rare Genetic Disease Patient Mutations
09:35 – 10:10	IL11	**P. Olejníková** (Slovakia): Microbial Resistance Is a Hot Topic – Are We Able to Slow Down the Adaptation Process of Microbial Cells and Thereby Combat the Increasing Resistance Phenomena?
10:10 – 10:30	OP13	**H. Bujdakova (**Slovakia): Antimicrobial Photodynamic Inactivation – A Promising Approach to Killing Infectious Pathogens
10:30 – 10:50	OP14	**A. Mahnic** (Slovenia): Microbiome Patterns and Biomarker Discovery in *Clostridioides difficile* Infection: Insights from Human Cohorts and *in Vitro* Models
10:50 – 11:10	Coffee break	
	Lobby bar	

11:10 – 12:25	**Session 5: Microbes in The Computer**	
	Chaired by: Bojan Žunar	
	Congress hall (Magnolia)	
11:10 – 11:45	IL12	**M. Shintani** (Japan): Behaviors of Plasmids in Different Natural Environments
11:45 – 12:05	OP15	**D. Balázs** (Hungary): Structure-Activity Relationships of *Trichoderma peptaibols* – A Crucial Step Towards Practical Applications
12:05 – 12:25	OP16	**A. Colautti** (Italy): Whole Genome Sequencing and Machine Learning Assisted Study to Understand Diffusion Dynamics of *Legionella pneumophila* in A Local Area
12:30	**Closing words**	
	Congress hall (Magnolia)	

IL	Invited lecture
OP	Oral presentation
SL	Sponsored lecture
TL	Tribune lecture

INVITED LECTURES

IL1

CO_2 Capture and Conversion to Base Chemicals by Engineered Autotrophic Yeast

Diethard Mattanovich[1,2]

[1]*University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Biotechnology, Institute of Microbiology and Microbial Biotechnology, Vienna, Austria*
[2]*acib GmbH, Vienna, Austria*

diethard.mattanovich@boku.ac.at

The methylotrophic yeast *Pichia pastoris* (*Komagataella phaffii*) is widely used in the manufacture of industrial enzymes and pharmaceuticals. Like most biotechnological production hosts, *P. pastoris* is heterotrophic and grows on organic feedstocks that have competing uses in the production of food and animal feed. In a step toward more sustainable industrial processes, we have described the conversion of *P. pastoris* into an autotroph that grows on CO_2 as its only carbon source. In addition to the synthetically engineered Calvin-Benson-Bassham cycle we have integrated the heterologous genes for lactic and itaconic acid synthesis. 13C labeling experiments proved that the resulting strains are able to produce organic acids via the assimilation of CO_2 as a sole carbon source. Further engineering attempts to prevent the lactic acid consumption increased the titers to 600 mg L^{-1}, while balancing the expression of key genes and modifying screening conditions led to 2 g L^{-1} itaconic acid. Bioreactor cultivations suggest that a fine-tuning of CO_2 uptake and oxygen demand of the cells is essential to reach a higher productivity. Further metabolic and process engineering toward the production of value-added bulk chemicals by microbial assimilation of CO_2 will be discussed in the light of sustainability of industrial bioprocesses.

IL2

rRNA Operon Multiplicity as a Bacterial Genome Stability Insurance Policy

Ivan Matic

Institut Cochin, INSERM U1016 - CNRS UMR8104 - Université Paris Cité, Paris, France

ivan.matic@inserm.fr

The ability to modulate translation capacity, which resides greatly on a number of ribosomes, provides robustness in fluctuating environments. Because translation is energetically the most expensive process in cells, cells must constantly adapt the rate of ribosome production to resource availability. This is primarily achieved by regulating ribosomal RNA (rRNA) synthesis, to which ribosomal proteins synthesis is adjusted. The multiplicity of rRNA encoding operons per bacterial genome exceeds requirements for the maximal growth rates in non-stress conditions. We provide evidence that a major function of rRNA operon multiplicity is to ensure that individual operons are not saturated by RNA polymerases during adaptation to environmental fluctuations, which can result in catastrophic chromosome replication failure and cell death.

IL3

Non-Conventional Yeasts for Biofuels and High-Value Chemicals

Andriy Sibirny[1,2]

[1]*Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine*
[2]*Institute of Biology and Biotechnology, University of Rzeszow, Rzeszow, Poland*

sibirny@yahoo.com

Non-conventional yeasts (yeasts different from *Saccharomyces cerevisiae*) often possess unique metabolic features which make them the promising organisms for basic and applied studies. The thermotolerant yeast *Ogataea polymorpha* grows and ferments major pentose sugar for lignocellulose, xylose, though ethanol yield and productivity in the wild-type strain is low. Using approaches of metabolic engineering and random selection, ethanol production from xylose at 45°C was increased for 50-fold exceeding 20 g/L. For this, gene *XYL1* coding for xylose reductase was engineered and overexpressed, additionally, native genes *XYL2*, *XYL3*, *DAS1*, *TAL2* were overexpressed while gene *CAT8* coding for transcription factor was deleted. Additionally, mutants resistant to anticancer drug 3-bromopyruvate and those growing on L-arabinose as sole carbon and energy source have been isolated. Simultaneous utilization and fermentation of glucose and xylose was achieved due to engineering and overexpression of *HXT1* gene coding for sugar transporter. Introduction in *O. polymorpha* of heterologous gene LDH from filamentous fungus *Rhizopus oryzae* led to the yeast strains accumulated significant concentrations of lactic acid in the media with both glucose r xylose (near 50 g/L). Flavinogenic yeast *Candida famata* was used to construct riboflavin, flavin mononucleotide and flavin adenine dinucleotide overproducers. Riboflavin production was activated due to overexpression of gene *RFE1* coding for putative riboflavin excretase, structural genes *RIB1* and *RIB6*, of transcription factor *SEF1* and genes involved in *GTP* (*PRS3*, *ADE4*) and ribulose-5-phosphate (*GND1*) oversynthesis. Overexpression of the bacterial genes *rosB*, *rosC* and *rosA* involved in biosynthesis of antibiotics roseoflavin, in riboflavin overproducing strains of *Komagataella phaffii* and *C. famata* resulted in yeast transformants accumulating high amounts of roseoflavin and aminoriboflavin. Further prospects of research and implementation of the achieved strains will be discussed.

IL4

Effect of Free Fatty Acids on *Streptomyces*

Branka Vasiljevic

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Srbia

brankav@imgge.bg.ac.rs

Members of the genus *Streptomyces* are renowned for their morphological complexity as well as their remarkable biosynthetic potential to produce an unrivalled range of important secondary metabolites, including half of all known clinically used antibiotics, not to mention other bioactive molecules – anticancer agents, antifungals, biocontrol agents, and immunosuppressors.

A novel aerobic, rapidly growing and sporulating strain *Streptomyces* sp. NP10 was isolated from the soil sample taken underneath decaying wood. The surface-grown culture was developing light yellow oily droplets on the surface of mature white spore chains. Analysis of the *Streptomyces* sp. NP10 genome revealed that it is closely related to several strains of *S. griseus*. Comparative analyses of secondary metabolite biosynthetic gene clusters allowed the identification of a cluster that is not common in *S. griseus*-related streptomycetes. A concurrent lipidomics study revealed a large structural diversity of free fatty acids (FAs) with over 50 FAs (C7-C30) identified from this strain.

FAs are omnipresent molecules that have roles in the host defence of many multicellular organisms, including mammals, against potential pathogenic or opportunistic microorganisms. it is well established that free FAs are one of the most active antimicrobial agents present on human skin and usually, their presence is sufficient to control the bacterial microbiota.

We were evaluating the effects of free FAs on the growth, morphology, and secondary metabolite production of four *Streptomyces* strains. We found that medium chain length FAs with 11 to 13 C atoms were most toxic to all tested *Streptomyces* strains, even in the concentration of 100 μg ml^{-1}. The effect of short-chain fatty acids (SCFA) of 4 and 5 C atom chain length proved to be complex. In liquid media, C4 SCFA could hinder while C5 SCFA could boost the production of a broad range of produced FFAs in *S. griseus* NP10. The presence of SCFA influenced the morphology of TK24 and NP10 submerged mycelium. When supplied in the vapour phase SCFA inhibited the growth of all tested *Streptomyces* strains on solid media. Vapours of C4 SCFA delayed undecylprodigiosin production in *Streptomyces* sp. JS520 while in *S. griseus* NP10 increased 4-fold total FFA production. Taken together, effects observed when low concentrations of SCFA were used, suggested their function as signalling molecules and not only as a precursor of fatty acid synthesis.

IL5

Enzyme Discovery and Protein Engineering for the Synthesis of New Biobased Polymers

Robert Kourist[1,2], Andrea Nigl[1,2], Kamela Myrtollari[1,3], Andreas Taden[3]

[1]*Austrian Centre of Industrial Biotechnology, Graz, Austria*
[2]*Graz University of Technology, Graz, Austria*
[3]*Henkel AG & Co. KGaA, Adhesive Research/Bioconjugates, Düsseldorf, Germany*

kourist@tugraz.at

The synthesis of renewable and sustainable polymeric materials as replacement of petroleum-based raw materials has been receiving increasing attention. Enzymes offer mild reaction conditions and often outstanding selectivity for the conversion of biobased precursor molecules into value-added chemicals. In this context, the biocatalytic synthesis of biobased olefins represents an important target due to their high relevance for the production of a multitude of polymeric materials.

The alpha-methylene lactone tulipalin A has two polymerizable functional moieties and is a potential substitute of (meth)acrylates in vinyl-addition polymerization and (co)monomer for lactone ring-opening polymerization. While tulipalin A can be isolated from the flowers of tulips and alstroemerias, its biosynthesis remains unknown. We propose a synthesis from isoprenyl acetate, which itself can be produced via the microbial hemiterpenoid metabolism. Selective hydroxylation of isoprenyl acetate in C4-position and subsequent oxidation of the intermediate hydroxy group gives rise to 4-acetoxy-2-methylene butyric acid, whose hydrolysis and cyclization then leads to tulipalin A. Rational design of a membrane-bound alkane-monooxygenase aiming to increase its activity in the selective hydroxylation of isoprenyl acetate will be presented.

Phenolic styrenes can be obtained by enzymatic decarboxylation of hydroxycinnamic acid, which are derived from the hemicellulose fraction of plants and are thus possible byproducts from the processing of corn crops, sugar beets, and rapeseed. Bacterial phenolic acid decarboxylase (PAD) catalyzes the decarboxylation under mild reaction conditions. While the activity of the enzyme is high, the low solubility of its substrates presents a severe limitations for industrial application. By ancestral sequence reconstruction (ASR), we obtained a decarboxylase variant which is significantly more thermostable (T_m = 77 °C) than the well-characterized PAD from Bacillus subtilis (T_m = 54 °C). Application of mixtures of natural deep eutectic solvents (NADES) and water led to a further stabilization of the enzyme. The new thermostable variant offers the potential to enhance the industrial application of the process by increasing both space-time yield and productivity.

IL6

Optimising *Bacillus subtilis* for Industrial Enzyme Production

Colin Harwood

Centre for Bacterial Cell Biology, Newcastle University, Newcastle upon Tyne, UK

colin.harwood@ncl.ac.uk

Bacillus subtilis and close relatives are among the most versatile and widely exploited industrial microorganisms. *Bacillus* spp. are used for the production of a variety of commercially important products, including industrial enzymes, vitamins, amino acids, and antifungal and antibacterial peptides. Analysis of this bacterium over more than 60 years has revealed detailed knowledge of its biochemistry, physiology and genetics, making it one of the most amenable host bacteria for synthetic biology/industrial biotechnology processes.

B. subtilis efficiently secretes native proteins and those from related bacteria at concentrations in excess of 20 g/L. However, yields of heterologous proteins, such as therapeutic proteins, are much more variable (µg – mg/L). To better understand the *Bacillus* secretion pathway, we have systematically studied the synthesis and secretion of extracellular proteins from the cradle (emergence from the ribosome) to the grave (release into the culture medium). In so doing, we have identified various pathway bottlenecks. With a clearer understanding of the secretion pathway, we have used a combination of synthetic biology and gene editing technologies to identify and overcome many pathway bottlenecks that limit productivity. This includes, on the one hand increasing the expression of genes encoding heterologous industrial proteins and, on the other hand, limiting the impact of quality control systems that detect and degrade foreign or misbehaving proteins.

IL7

Yeast Sterol Engineering

Holly Stolterfoht-Stock[1], Melanie Bäck (Hirz)[2], Cleiton Martins Souza[3], Tamara Wriessnegger[1], Martin Lehmann[4], Barbara Petschacher[1], Howard Riezman[3], Harald Pichler[1,2]

[1]*Austrian Centre of Industrial Biotechnology, Graz, Austria*
[2]*Institute of Molecular Biotechnology, Graz University of Technology, Austria*
[3]*Institute of Biochemistry, University of Geneva, Switzerland*
[4]*Solvias AG, Kaiseraugst, Switzerland*

harald.pichler@tugraz.at

Yeasts use ergosterol as the main sterol in their membranes but tolerate certain modifications to the sterol structure and abundance. Viable sterol-engineered strains may show distinct phenotypes that can be employed for studying the roles of sterols in diverse cellular transport and signaling processes.
Moreover, modified yeast sterols are of commercial interest for pharmaceutical, cosmetic as well as food and feed applications. Yeast strains with engineered sterol patterns, eg. producing vitamin D3 precursor 7-dehydrocholesterol (7-DHC), can readily be generated. However, baker's yeast sequesters a surplus of sterols as steryl esters in lipid particles. As the two sterol acyltransferases of *S. cerevisiae*, Are1p and Are2p, sequester virtually every possible sterol molecule including early precursors like zymosterol, the overall 7-DHC yield is limited by the lack of sterol-specificity of sterol acyltransferases. We have evolved Are2p employing an unprecedented screening assay and have characterized Are2p variants that esterify 7-DHC with increased specificity. We managed to pinpoint a number of amino acid exchanges that influence overall Are2p activity, Are2p sterol specificity and both. Enzyme evolution and subsequent shuffling experiments are bringing us closer in understanding structure-function relationships of sterol acyltransferases.

IL8

Genomic Adaptations of *Saccharomyces* Yeasts to Brewing Environments

Neža Čadež

Biotechnical faculty, University in Ljubljana, Ljubljana, Slovenia

neza.cadez@bf.uni-lj.si

The use of bottom fermentation to produce lager beer most likely began in the 16th century in Bavarian monastery breweries. The reason for developing a new type of beer was probably the low fermentation temperature, which was initially intended to reduce contamination and the formation of undesirable aromas. However, this cold brewing environment was a key ecological factor that triggered the unusual biological solution, namely hybridization between two closely related *Saccharomyces* species that formed an ancestor of today's lager yeast strain. Old brewing practice of "back-slopping" led to further genomic rearrangements leading to an efficient industrial strain as we know it today. Recent advances in sequencing technologies have allowed us to assemble the genome of the Weihenstephan 34/70 strain, which is a hallmark of Central European breweries. In addition, we performed experiments to analyse the genomic adaptation of this strain during 30-cycle repitching. Detailed analyses of these genetic changes revealed that the yeast biomass is genetically heterogeneous and subject to evolutionary dynamics. By comparing the genetic variants along the time course, we found that the structure of the population is constantly changing, and the final result (the beer) is the sum of all these differences between clones. This knowledge is now being used to construct novel brewing strains adapted to new substrates for efficient conversion of sugars to ethanol. One such case will also be discussed.

Financial support: The work has been supported by Laško-Union d.d. brewery and Slovenian Research Agency (ARRS L4-8222).

IL9

Exploitation of Interkingdom Microbial Interactions in Bioenergy Generation (Algal-Bacterial Interactions for Biohydrogen Production and Complex Microbial Interactions in Anaerobic Digestion)

Roland Wirth[1,2], Zoltán Bagi[2], Prateek Shetty[1], Márk Szuhaj[2], Sally Cheung[2], Attila Farkas[1], Bernadette Pap[1], Kornél L. Kovács[2,3], Gergely Maróti[1,4]

[1]*Institute of Plant Biology, Biological Research Centre, Szeged, Hungary*
[2]*Department of Biotechnology, University of Szeged, Szeged, Hungary*
[3]*Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry, University of Szeged, Szeged, Hungary*
[4]*Seqomics Biotechnology Ltd., Mórahalom, Hungary*

maroti.gergely@brc.hu

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 biostimulant products. Pairwise algal-bacterial combinations were cultivated for five days in synthetic wastewater. The accumulated biohydrogen was recorded and the specific algal growth rate was determined based on the variation in algal cell concentration. Successful bacterial candidates were identified by high algal biohydrogen production and increased algal biomass. We have investigated the effect 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.

IL10

Humanization of Yeast *Saccharomyces cerevisiae* to Study Human Proteins and Rare Genetic Disease Patient Mutations

Bruno Rinaldi, Séverine Bär, Sylvie Friant

University of Strasbourg, Génétique Moléculaire, Génomique, Microbiologie (GMGM), Strasbourg, France

s.friant@unistra.fr

Some patients with rare genetic diseases (myopathy, neuropathy, ciliopathy, blood diseases) do not have mutations in the known genes and exome or genome sequencing reveals new genetic targets. In our research team, we are using different cell models including yeast *Saccharomyces cerevisiae* to validate these new genes and study their variants responsible for rare diseases. Indeed, these mutations often detected in a small number of patients, are the most difficult to validate and an approach starting with the use of the yeast model is not expensive and fast. We are creating humanized yeast cells expressing the human cDNA (tissue-specific isoform) either wild-type or bearing the patient mutation revealed by the sequencing data. Different phenotypes and pathways as membrane trafficking, autophagy, or phosphoinositides lipids levels were analyzed in these humanized yeasts to determine the defects linked to the mutation. The yeast model has proven to be very effective so far, as humanization of yeast allowed us to validate new genes in rare diseases and to determine at the cellular level the defects due to the patient mutations.

IL11

Microbial Resistance Is a Hot Topic – Are We Able to Slow Down the Adaptation Process of Microbial Cells and Thereby Combat the Increasing Resistance Phenomena?

Petra Olejníková, Ján Víglaš

Institute of Biochemistry and Microbiology, Faculty of food and chemical technology, Slovak University of Technology in Bratislava, Bratislava, Slovakia

petra.olejnikova@stuba.sk

Microbial resistance is a worldwide problem that is of interest for many research groups. Many years ago, bacterial resistance came to the forefront of the scientific field and still holds the first place. Together with the medical progress in therapy also appeared the resistance of fungal cells, which is gaining importance, catching up the primacy of bacterial cells in the field of resistance. Since the fungal infections are frequent in immunocompromised patients, the higher attention is paid to the mechanisms of fungal resistance, while physiological changes that precede the resistant phenotype remain elusive. Thus, many questions are still open in this field of research, and no clear answer has been defined yet - that is the reason why we are looking into fungal adaptation mechanisms as a reaction to the presence of antifungals.

Considering the mode of action of antifungal compounds, most of them are targeting the surface structures of fungal cells such as ergosterol (azoles, polyenes, allylamines, morpholines…) or the synthesis of fungal cell wall (echinocandins). Exposure of the fungus to antifungal agents causes chemical stress leading to induction of signalling pathways conserved through the fungal kingdom resulting in the mechanisms enabling compensation of the caused damages. This leads to tolerance that usually follows with the induction of resistance mechanisms to antifungal compounds. The compounds of our interest were the azoles (the most used antifungal compounds in clinical practice). In our experimental work, we have followed the response of *Neurospora crassa* after exposure to various azole structures, e.g., fluconazole, voriconazole, ravuconazole, ketoconazole and prochloraz. Our results have shown that even *N. crassa*, though not being a fungus that demonstrates the resistance phenotype, is able to respond to antifungal agents in similar ways like fungi showing inducible resistance mechanisms. Moreover, we have shown that the response of the fungus includes a wide range of compensation mechanisms, reaching beyond those currently associated with resistance to antifungal compounds.

This work was supported by the Grant Agency of Ministry of Education, Science, Research and Sport of the Slovak Republic, research project No. VEGA 1/0388/22 and by the Slovak Research and Development Agency, under the Contract No. APVV-19-0.

IL12

Behaviors of Plasmids in Different Natural Environments

Masaki (Masa) Shintani[1,2,3]

[1]*Applied Chemistry and Biochemical Engineering Course, Department of Engineering, Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka, Japan*
[2]*Research Institute of Green Science and Technology, Shizuoka University, Shizuoka, Japan*
[3]*Japan Collection of Microorganisms, RIKEN BioResource Research Center, Ibaraki, Japan*

shintani.masaki@shizuoka.ac.jp

Plasmids are ones of the mobile genetic elements to transmit different genes including antibiotic resistance genes and thus, it is important to understand which plasmids are truly spread in nature. Various conjugative plasmids were obtained by exogenous plasmid capture, biparental mating, and/or triparental mating methods from different environmental samples [1]. Several novel plasmids were obtained, which were related to IncP/P-1 plasmids but compatible with one of IncP/P-1 plasmids. Some plasmids could be classified into novel subgroups of IncP/P-1 plasmids. There were two large clades in IncP/P-1 plasmids, clade I and II. Plasmids in clade I and II included a variety of antibiotic resistance genes. Nucleotide compositions of newly found plasmids showed different tendencies compared to the previously well-studied IncP/P-1 plasmids, and indeed, the host range of plasmids of clade II was different from that of clade I. Many of the PromA plasmids, including PromAα, PromAβ, -γ, and -δ subgroups, were obtained as many as IncP/P-1 plasmids. The host ranges of these PromA group plasmids were broad and they could be transferred to different and distinct classes of *Proteobacteria*. Surprisingly, the obtained PromA plasmids do not carry any previously known accessory genes. These findings indicate the presence of 'hitherto-unnoticed' conjugative plasmids in nature, which would have important roles in the exchange of various genes, including antibiotic resistance genes, among different bacteria.

[1] Hayakawa et al., 2022, Applied and Environmental Microbiology, 88:e01114-22.

ORAL PRESENTATIONS

OP1

Secondary Metabolites from Mono- And Co-Cultures of Bacteria Associated with the Freshwater Bryozoan *Cristatella mucedo*

Inmaculada Tocino-Márquez[1,5], Martin Zehl[2], Petra Pjevac[3,4], Rasmus Kirkegaard[3,4], Mathias Flieder[3,5], Alexander Loy[3,4,5], Thomas Rattei[3,5], Sergey B. Zotchev[1]

[1]*Department of Pharmaceutical Sciences, Division of Pharmacognosy, University of Vienna, Austria*
[2]*Mass Spectrometry Centre and Department of Analytical Chemistry, University of Vienna, Austria*
[3]*Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria*
[4]*Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna, Austria*
[5]*Doctoral School in Microbiology and Environmental Science, University of Vienna, Austria*

inmaculada.tocino.marquez@univie.ac.at

In nature, bacteria usually exist as part of diverse microbial communities where different types of interactions occur. Studying well-defined microbial interactions and their metabolic activities has led to the discovery of new bioactive secondary metabolites [1]. Recent advances in bacterial genomics and in understanding the secondary metabolite biosynthesis pathways and co-cultivation techniques revealed the potential of co-cultures to produce compounds that could not be produced by single isolates in monocultures [2]. These biosynthetic pathways are encoded by specialized regions of bacterial genomes called biosynthetic gene clusters (BGCs), most of which are "silent" under laboratory conditions, and the environmental signals triggering their expression remain largely unknown. In this study, we explore the biosynthetic potential of the bacterial microbiota of a freshwater bryozoan *Cristatella mucedo*. Representatives of 29 bacterial genera were isolated from this bryozoan, taxonomically classified, and some of them genome-sequenced. The isolates with the highest number of BGCs, and hence the capacity to produce secondary metabolites, were predicted using antiSMASH software [3]. The mono-cultivation of *Micromonospora* sp. resulted in the identification of a potentially novel xanthone natural product with anti-bacterial activity. Co-cultivation of *Rhodococcus* sp. with *Bacillus* spp. isolates, followed by HPLC and LC-MS analysis, revealed the induction of several potentially new compounds, one of which was preliminary identified as a product of specific non-ribosomal peptide synthetase from *Bacillus* sp. In addition, two ribosomally synthesized peptides produced by *Rhodococcus* sp. were identified, which are degraded by co-cultured Bacillus sp. Further work on these peptides and other secondary metabolites from the bryozoan bacterial isolates aimed at characterization of their structures and bioactivity is ongoing.

[1] Bertrand S, Bohni N, Schnee S, Schumpp O, Gindro K., & Wolfender JL (2014). Metabolite induction via microorganism co-culture: A potential way to enhance chemical diversity for drug discovery. Biotechnology Advances, 32(6), 1180–1204.
[2] Traxler MF, Watrous JD, Alexandrov T, Dorrestein PC, Kolter R. (2013). Interspecies interactions stimulate diversification of the Streptomyces coelicolor secreted metabolome. mBio, 4(4): e00459-13.
[3] Kai Blin, Simon Shaw, Alexander M Kloosterman, Zach Charlop-Powers, Gilles P van Weezel, Marnix H Medema, & Tilmann Weber.

OP2

Impact of Algal-Bacterial Co-Cultivation on Algal Biomass and Biohydrogen Production

Prateek Shetty, Bernadette Pap, Gabriella Terhes, Attila Farkas, Vaishali Rani, Gergely Maróti

Biological Research Center, Hungary

prateekshettys@gmail.com

Past empirical research has demonstrated that bacterial association is essential for enhancing algal growth, algal biohydrogen generation, and bioremediation. However, little is known about the mechanisms and microalgal functions activated under bacterial associations.

To investigate this, different bacterial species were cultivated with *Chlamydomonas reinhardtii* cc124. Bacterial species were isolated from diverse environments, including biogas sludge, soil, and commercial biostimulants. Pairwise algal-bacterial combinations were cultivated for five days in 25x synthetic wastewater media. The accumulated biohydrogen was recorded daily, and the specific algal growth rate was determined based on the variation in algal cell concentration. Successful bacterial candidates were identified by high algal biohydrogen production and algal biomass.

All bacterial interactions contributed to an increase in algal biomass. Microalgal Chlorophyll a/b ratio, accumulated lipids, and accumulated carbohydrates in *C. reinhardtii* cc124 were enhanced by co-cultivation with bacterial partners. We found that members of the *Bacillaceae* family had a species-specific impact on the improvement of algal biohydrogen generation in *C. reinhardtii* cc124 and other algal species, such as *Chlorella* sp. and *Micractinium* sp. *C. reinhardtii* cc124 exhibited distinct physiological responses to bacterial association. When *C. reinhardtii* cc124 was cultured with *Bacillus* sp., an accumulation of algal lipids was seen, and when it was cultivated with *Methylobacterium* sp., an accumulation of carbohydrates was observed. Finally, a transcriptome analysis was carried out to specifically study the algal gene expresssion under bacterial association. Genes upregulated under bacterial co-cultivation revealed enrichment of Salicylic acid biosynthetic and metabolic process and glycoprotein biosynthesis.

OP3

Comparison of Bioremediation Strategies for the Biodegradation of Microplastics by Bacteria and Yeast

Martina Miloloža, Kristina Bule Možar, Šime Ukić, Tomislav Bolanča, Dajana Kučić Grgić

Faculty of Chemical Engineering and Technology University of Zagreb, Croatia

miloloza@fkit.hr

Plastics have excellent application properties and therefore are used in various fields of human life. The annual production of plastics is increasing, as is the amount of plastic waste. Plastics are ubiquitous in the environment, where they break down into smaller particles called microplastics (MP) due to physical impact, UV radiation, and other environmental factors. Recently, ecotoxicological studies for MP have revealed potential adverse effects of MP on aquatic organisms. Therefore, it is necessary to investigate possible solutions to remove MP from the environment. In this context, the biodegradation of PS and PVC MP was studied using different microorganisms isolated from the environment enriched with MP. The experiments were divided into two parts: preliminary experiments and main experiments. The preliminary experiments on biodegradation of MP were performed with 5 bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas alcaligenes*, *Delftia acidovorans* and *Bacillus licheniformis*) and 5 yeasts (*Saccharomyces cerevisiae*, *Rhodotorula g*lutinis, *Geotrichum c*andidum, *Trichosporon* sp. and *Candida parapsilosis*). During 30 days, the changes in the total number of bacteria and yeasts (CFU) were monitored. In addition, the particles of MP were analyzed by FTIR spectroscopy. Based on the results of the preliminary experiments, the most effective bacterium and yeast were selected for the main biodegradation experiment. Therefore, the main experiments with *Delftia acidovorans* and *Candida parapsilosis* were conducted according to a full factorial experimental design with 3 parameters at 3 levels to determine the optimal process conditions. The parameters studied were pH-value, agitation speed, and optical density of the bacteria/yeast suspension. In this case, the size of MP (25-100 μm), the concentration of MP (500 mg/L), and the temperature (25 ± 0.2 °C) were constant. In addition, the main objective of this work was to determine the most efficient bacterium and yeast for biodegradation of MP and the optimal conditions to achieve the highest efficiency of the biodegradation process.

OP4

The Potential of New *Paraburkholderia* sp. Isolates for Upcycling Lignin from Invasive Plant Species

Maja Zugan[1,2], Helena Plešnik[1,2], Dmitrii Deev[1,2], Lidija Strojnik[1], Tina Kosjek[1], Nives Ogrinc[1], Tomaž Rijavec[1], Aleš Lapanje[1]

[1]*Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia*
[2]*International Postgraduate School Jožef Stefan, Ljubljana, Slovenia*

maja.zugan@ijs.si

Invasive alien plant species (IAPS) pose a significant threat to global ecosystems. Current efforts are mainly focused on removing and eradicating IAPS, but little attention has been paid to utilizing their biomass. However, due to their higher lignin content compared to native plant species and the great potential of lignin as a renewable source of aromatic and other organic compounds, IAPS have the potential to be used for production of various valuable compounds of industrial importance. In this study, we present a case for the production of value-added compounds from IAPS using bacterial soil isolates. Lignin degrading bacterial isolates were acquired from IAPS rhizosphere and characterized by 16S rRNA, genome sequencing, lignin degrading enzyme assays and measurements of degraded lignin and lignin in the IAPS plant biomass. To characterize the metabolic degradation of lignin, GC-MS and targeted LC-MS were performed, where 8 lignin degradation compounds were measured. Isolated bacteria were characterized as *Paraburkholderia* sp. and genomic analysis showed the presence of 14 enzyme encoding genes involved in various parts of lignin degradation. Furthermore, *Paraburkholderia* sp. strain L4 was able to degrade lignin from the IAPS plant biomass as well as lignin from black liquor of processed IAPS. GC-MS and LC-MS analysis of degradation products showed metabolic activity of the bacteria, where some compounds spontaneously released from lignin were consumed, while others such as 4-hydroxycinnamic acid, ferulic acid and 4-hydroxyacetophenone were produced. These data were supported by the presence of the corresponding enzyme encoding genes. Our results showed that the potential of IAPS utilization for lignin transformation into value-added compounds can open new possibilities for exploitation of IAPS through involvement of specific microorganisms.

OP5

Genome Mining of *Streptomyces* sp. S4.7 for Meroterpenoids and Prenylated Phenazines

Olha Schneider[1], Martin Zehl[2], Sergey B. Zotchev[1]

[1]*Division of Pharmacognosy, Department of Pharmaceutical Sciences, Faculty of Life Science, University of Vienna, Vienna, Austria*
[2]*Mass Spectrometry Centre & Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria*

sergey.zotchev@univie.ac.at

Streptomyces is a bacterial genus known for its ability to produce a wide variety of bioactive secondary metabolites produced through intricate biosynthetic pathways encoded by Biosynthetic Gene Clusters (BGCs). *Streptomyces* species have complex genomes containing multiple BGCs, allowing them to produce a diverse array of secondary metabolites with diverse biological activities, such as antimicrobial, antitumor, or immunosuppressive. In this study, we used genome mining to discover potentially new compounds from *Streptomyces* sp. S4.7. The strain was isolated from the rhizosphere of a *Leontopodium nivale* (Edelweiss) collected in Austria and was shown earlier to produce new cyclic lipopeptides viennamycins. Heterologous expression of the potentially interesting hybrid BGC of *Streptomyces* sp. S4.7 predicted to specify biosynthesis of mero terpenoids marfuroquinocins and prenylated phenazines was performed in genetically engineered *Streptomyces coelicolor* M1154. The production of marfuroquinocins was achieved only upon co-expression of this BGC with the gene for pathway-specific regulators, leading to the discovery of a new marfuraquinocin congener with antibacterial activity. However, the heterologous expression did not result in the production of prenylated phenazines, indicating an independent regulatory pathway from mero terpenoids. Attempts on activation of the prenylated phenazines biosynthetic pathway are being pursued using various approaches based on the CRISPR-Cas9 genome editing system.

OP6

Yeasts as Biotechnological Producers of Bacterial Antibiotics Roseoflavin and Aminoriboflavin

Justyna Ruchala[1], Kostyantyn Dmytruk[2], Liubov Fayura[2], Olena Dmytruk[2], Daria Fedorovych[2], Olena Motyka[3], Grzegorz Chrzanowski[1], Andriy Tsyrulnyk[2], Yuliia Andreieva[2], Hans Marx[4], Diethard Mattanovich[4], Andriy Sibirny[1,2]

[1]*University of Rzeszow, Poland*
[2]*Institute of Cell Biology National Academy of Sciences of Ukraine, Ukraine*
[3]*Research Institute of Epidemiology and Hygiene of the Danylo Halytsky Lviv National Medical University, Ukraine*
[4]*University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Microbiology and Microbial Biotechnology, Austria*

jruchala@ur.edu.pl

Soil bacteria *Streptomyces davaonensis* and *Streptomyces cinnabarinus* synthesize a promising broad-spectrum antibiotic roseoflavin; its synthesis starts from flavin mononucleotide and proceeds through an immediate precursor, aminoriboflavin, that also has antibiotic properties. Roseoflavin accumulation by the natural producers is rather low, whereas aminoriboflavin accumulation is negligible. Yeasts have many advantages as biotechnological producers relative to bacteria; however, no recombinant producers of bacterial antibiotics in yeasts are known. The *rosB*, *rosC*, and *rosA* roseoflavin biosynthesis genes have been expressed in riboflavin- or flavin mononucleotide overexpressing strains of *Candida famata* and *Komagataella phaffii* (*Pichia pastoris*). Both recombinant yeast species accumulated aminoriboflavin, whereas only the latter produced roseoflavin. The accumulation of aminoriboflavin and roseoflavin by the recombinant strain of *K. phaffii* in a bioreactor reached 22 and 130 mg L^{-1}, respectively. For comparison, recombinant strains of the native bacterial producer *S. davaonensis* accumulated about one order less of roseoflavin, while no recombinant producers of aminoriboflavin were reported. Yeast strains, in addition to antibiotics, also overproduced riboflavin. It is interesting to note that most of riboflavin and antibiotics have been synthesized and accumulated in the cultural medium after growth cessation, i.e., in the stationary growth phase. Aminoriboflavin isolated from recombinant strains of *C. famata* inhibited growth of pathogenic bacteria *Staphylococcus aureus* (including MRSA) and *Listeria monocytogenes*.

OP7

New Approach for Development of Artificial Microbial Consortium for Biological Pretreatment of Lignocellulosic Biomass

Quang D. Nguyen[1], Vi N.H. Vu[1], Csilla Kohari-Farkas[1], Erika Bujna[1], Akos Kilin[1], Gabor Laszlovszki[1], Robert Filep[1], Vijai Kumar Gupta[2]

[1]Department of Bioengineering and Alcoholic Drink Technology, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary
[2]Biorefining and Advanced Materials Research Center, Scotland's Rural College (SRUC), Edinburgh, United Knigdom

nguyen.duc.quang@uni-mate.hu

Lignocellulosic biomass is biggest renewable raw material in the world. This is complex polymer structure cellulose, hemicelluloses and lignin, as well as other components in small amounts, which are associated with non-covalent bonds and covalent cross-linkages. Due to the recalcitrant lignocellulosic matrices, pretreatment is one of key processes in the utilisation of this type of biomass. There are different pretreatment approaches such as physical, chemical, physicochemical, biological, and combined methods. Among these routes, biological pretreatment has many advantages such high-cost effectiveness, lower energy requirements, mild conditions and chemical-free/environmental friendly technology. But biological pretreatment still faces some drawbacks such as low degradation efficiency, long process duration, and the risks of carbohydrate loss. To overcome this issues, microbial consortium with the synergistic action of microorganisms could be developed and used. In this study, new approach for construction and development of microbial consortium for pretreatment of lignocellulosic biomass were reported. This approach does not focus only one-one property of microorganisms involved, instead, it tries to take consideration of any roles of microbes in the consortium. First, the roles needed are figured out and then appropriate microbe with relevant criteria is involved into the construction of consortium. The new approach was successfully applied in the design and construction of different artificial microbial communities with 5-7 members for pretreatment of wheat bran and wheat straw with very high efficiency.
Our work was supported by Ministry of Technology and Industry Hungary under grant no.: KEHOP-3.2.1-15-2021-00037. Vu, Kilin, Laszlovszki and Filep are PhD students at Doctoral School of Food Science

OP8

Differential Abundance Analysis of "Core" Bacteriobiota During Key Growth Stages of Maize

Ivica Dimkić[1], Aleksandra Jelušić[2], Tamara Janakiev[1], Dunja Bandelj[3], Slavoljub Vukićević[4], Matjaž Hladnik[3]

[1]*Chair of Biochemistry and Molecular Biology, University of Belgrade - Faculty of Biology, Belgrade, Serbia*
[2]*Department of Life Sciences, University of Belgrade - Institute for Multidisciplinary Research, Belgrade, Serbia*
[3]*Department of Applied Natural Sciences, University of Primorska - Faculty of Mathematics, Natural Sciences and Information Technologies (UP FAMNIT), Koper, Slovenia*
[4]*Fertico D.O.O., Nitrogenous Fertilizer Manufacturing Enterprise, Indjija, Serbia*

ivicad@bio.bg.ac.rs

Numerous microbial taxa are used as biofertilizers because they have the potential to competitively colonize the rhizosphere or root interior of plants and promote nutrient uptake. The aim was to investigate the shifts of keystone bacterial species in maize roots and rhizosphere during seedling stage, flowering, and harvest, under different treatments of bacterial phytobiotic (PHY), poultry manure (PM) and their combination (PHY_PM). Soil bacterial communities remained largely unchanged regardless of the treatment applied or phenophase studied, with uncultured *Gaiellales* and *Bacillus* being the most abundant. In contrast, bacterial communities in roots differed in terms of distribution and relative abundance (RA) of different taxa between growth stages and treatments. The most abundant bacterial taxa in the roots during the initial seedling stage was *Pseudomonas*. In the flowering, *Bacillus* occurred with a two- to threefold higher RA in treatments with PHY or PM compared to the negative control, while *Lechevalieria* dominated during the harvest. Differential abundance analysis at the seedling stage showed a reduction of Pseudomonas in roots treated with PHY, while *Pseudonocardia* was significantly more abundant in roots treated with PM than in the other samples. *Massilia*, *Streptomyces*, *Lechevalieria*, *Microbacteriaceae*, *Aeromicrobium*, *Sphingomonas* and *Roseiflexaceae* were significantly higher in the PHY_PM treated root samples. *Steroidobacter* and *Bdellovibrio* were absent in PHY and PM treated root samples during flowering, while *Bdellovibrio* was significantly reduced in PHY_PM treatment compared to the negative control. RA of *Bradyrhizobium* and *Polaromonas* was reduced in PHY treated root samples, and *Dongia* was completely absent. *Gaiellales* was significantly more abundant, while *Sphingomonas* was less abundant in roots treated with PM and PHY_PM. *Solirubrobacter* and *Bdellovibrio* were absent from

roots during harvest in all three treatment types. *Pajaroellobacter* was completely absent in PHY and PM treatments, while *Steroidobacteraceae* were present only in PHY and *Cyclobacteriaceae* in PM. *Mucilaginibacter calamicampi* showed significantly higher RA in PHY_PM treatment, while *Sphingomonas* was reduced. Considering the non-disruptive effect of PHY on the "core" bacteriobiota and the positive effects on the presence of beneficial bacterial genera, such products could be proposed as a promising alternative to chemicals and organic fertilizers in maize cultivation.

OP9

Assessing the Health of the Marine Environment by Tracking a Complex Network of Microbial Communities in Sediments

Ivana Babić[1], Ana Ramljak[1], Jurica Žučko[2], Martina Furdek Turk[1], Dimitrios Karpouzas[3], Sotirios Vasileiadis[3], Mavro Lučić[1], Slavica Matijević[4], Maja Fafanđel[1], Nikolina Udiković Kolić[1], Ines Petrić[1]

[1]*Institute Ruđer Bošković, Zagreb, Croatia*
[2]*Faculty of Food Technology and Biotechnology, Zagreb, Croatia*
[3]*University of Thessaloniki, Greece*
[4]*Institute of Oceanography and Fisheries, Split, Croatia*

ibabic@irb.hr

Problem of multiple pollutants effecting marine ecosystems is one of the pressing questions in ecology and conservation. Especially impacted are the areas such as the Mediterranean basin, including the Adriatic coast, where more than 50% of the total population resides in its coastal zones, making it one of the most human-impacted environments. Microbial communities inhabiting sediment of coastal habitat play an important role in the material cycling and energy flow of the marine ecosystem. Microbial community structure may adapt to environmental stressors, especially by weakening the impact of pollutants discharged from human activities, and therefore should be represented as ecological status indicator. However, ecotoxicological tools and ecological indices based on microbial community structure and composition proposed to evaluate the environmental health are still in the infancy. Here, physicochemical characterization of the sediments (Microtox test, granulometry, TBT, metals, Hg, TOC, TON, P etc.) were combined with molecular-based approaches (amplicon and shotgun sequencing, qPCR target analysis) to find the missing link between anthropogenic disturbance and changes within sediment microbial assemblages. More than 60 different sediments were collected within seven harbors along the eastern Adriatic coast, being labelled as "hot spots" with poor or bad water and sediment quality and non-satisfying chemical and ecological status. The main aim was to highlight the significance of microbial communities in monitoring and consequently preserving the quality of marine environment, as part of Project MicroLink, funded by the Croatian Science Foundation. Microbial communities' structure, composition and diversity were evaluated on multi-domain level (bacteria, fungi and protists) using diversity indices, relative abundance and correlation analysis with the aim to discern patterns and trends in contaminated vs control locations. Evaluation of environmental health should also embrace sediment microbial community (structure and functions), as microbes are known as key players in controlling structure and functioning of the whole marine network.

OP10
Extended-Spectrum SS-Lactamase- And Carbapenemase-Producing *Enterobacterales* in Hospital Wastewater in Croatia

Ana Puljko[1], Svjetlana Dekić Rozman[1], Ivan Barišić[2], Ana Maravić[3], Marko Jelić[4], Ivana Babić[1], Nikolina Udiković Kolić[1]

[1]*Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia*
[2]*Molecular Diagnostics, Austrian Institute of Technology, Vienna, Austria*
[3]*Department of Biology, Faculty of Science, University of Split, Split, Croatia*
[4]*Department of Clinical Microbiology, University Hospital for Infectious Diseases, Zagreb, Croatia*

apuljko@irb.hr

Extended-spectrum ß-lactamase (ESBL)- and carbapenemase-producing *Enterobacterales* cause hospital outbreaks worldwide. A major route of transmission for these, often multidrug-resistant bacteria, into the aquatic environment is hospital wastewater. In this study, we characterized ESBL- and carbapenemase-producing enterobacterial isolates from two hospital wastewater in Zagreb. Bacterial colonies from chromogenic screening media were selected for species identification by MALDI-TOF MS. Antibiotic susceptibility testing and the production of ESBLs and carbapenemases were phenotypically assessed. ESBL and carbapenemase genes were detected by PCR and sequencing of selected amplicons or by whole genome sequencing (WGS). The molecular epidemiology of the isolates was assessed by pulsed-filed gel electrophoresis (PFGE) and multilocus sequence typing (MLST).
Of 200 isolates, 159 were confirmed as *Enterobacterales*, mainly *Escherichia coli*, *Citrobacter* spp., *Enterobacter cloacae* clonal complex (cplx), and *Klebsiella* spp. These isolates (69 ESBL- and 90 carbapenemase-producing) were multidrug resistant. The most prevalent ESBL genes among ESBL-producing isolates were *blaCTX-M-15* and *blaTEM-116*, whereas, among carbapenemase-producing isolates, *blaKPC-2* and *blaNDM-1* were most frequently detected, followed by *blaOXA-48*. Clinically relevant sequence types were found in 6 *E. coli*, 3 *Klebsiella pneumoniae*, and 5 *Enterobacter cloacae* cplx PFGE clusters. WGS revealed an association of resistance genes with mobile genetic elements, suggesting a possible mobilization of these genes.
To conclude, hospital wastewater can serve as a potential secondary reservoir for clinically important pathogens and resistance genes and therefore requires effective pretreatment before discharge into the municipal wastewater system.

Keywords: Carbapenemase, ESBL, hospital wastewater, antibiotic resistance, *Enterobacterales*

OP11

Phylogenetic Analysis of Indigenous Rhizobial Strains Nodulating Soybean (*Glycine max* L.) and Their Symbiotic Efficiency Under Drought Conditions

Sanja Kajić[1], Aregu Aserse[2], Kristina Lindström[2], Ivana Rajnović[1], Ivan Pejić[3], Sanja Sikora[1]

[1]*Department of Microbiology, University of Zagreb Faculty of Agriculture, Croatia*
[2]*Ecosystems and Environment Research programme, Faculty of Biological and Environmental Sciences and Helsinki Institute of Sustainability Science (HELSUS), Finland*
[3]*Department of Plant Breeding, Genetics and Biometrics, University of Zagreb Faculty of Agriculture, Croatia*

skajic@agr.hr

Symbiotic associations between legumes and specific group of soil bacteria enable considerable entries of biologically fixed nitrogen into the soil. The rhizobia inoculation of soybean is a sustainable practice to induce atmospheric nitrogen fixation and subsequently improve crop productivity and soil fertility. The main advantage of indigenous rhizobial strains relates to their high competitiveness and adaptation ability to specific environmental conditions. Drought is one of the most important factors limiting nitrogen fixation, growth and yield of soybean. In this study, 60 indigenous rhizobia isolated from 29 different regions of Croatia were subjected to in vitro investigations of different water conditions. Based on the results obtained by the RAPD method, sequencing of the 16S rRNA gene and in vitro drought test representative strains were selected for further analysis of sequences *rpoB, glnII, gyrB, nodC* and *nifH* gene. Phenotypic characterization of isolates included testing the tolerance to unfavorable soil conditions intrinsic antibiotic resistance, biochemical characterization, assimilation of different carbon sources, generational time and screening for plant growth promoting properties (PGPR). Greenhouse experiment was setup using two factors on the basis of a completely randomized block design. The 16S rRNA results showed that 75% strains from this study belong to genus Bradyrhizobium. Phylogenetic analysis of the three housekeeping genes *rpoB, gyrB* and *glnII* showed that the strains were identified as *B. diazoefficiens* and *B. ottawaense* species for the first time in Croatian soils and species *B. japonicum* identified earlier. The results of the *nodC* gene analysis of indigenous rhizobial strains isolated from Croatian soils showed that all of them belong to the symbiovar glycinearum. The phylogeny of most *nifH* genes coincides with the phylogeny of the *nodC* gene indicating the co-evolution of these two genes. PGPR characterization of isolates showed their significant variability. Results of greenhouse experiment showed that the hightest

nodule number was obtained when soybean was inoculated with indigenous strain S1/5 (*B. japonicum*). Nodule dry weight is parameter indicating nodulation capacity of strain. Strains S3/5 (*B. japonicum*) S25/2 (*B. diazoefficiens*) S32 (*B. ottawaense*) and S37 (*B. japonicum*) showed significant tolerance to unfavourable conditions and PGPR characterization.

OP12

Analysis of Microbial Community and Specific Resistance Genes of the Microplastics, Water and Surroundings of Danube River

Magdaléna Rusková, Jelena Pavlović, Zuzana Farkas, Nikola Klištincová, Francesca Maisto, Mária Bučková, Andrea Puškárová, Domenico Pangallo

Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia

magdalena.kapustova@savba.sk

Antibiotic-resistant bacteria (ARB) are a risk for patients and healthy people. The indiscriminate use of antibiotics has led to the development of multidrug-resistant bacteria. Large amounts of antibiotics are released into municipal wastewater due to incomplete metabolism in humans or due to the disposal of unused antibiotics, which finally find their way into different natural environmental compartments [1]. Especially microplastics in different environments provide a specific place for the bacterial community. Biofilms on microplastics facilitate cell-to-cell communication and transfer of antimicrobial resistance genes (ARGs). Moreover, ARB in the environment can transfer their antibiotic-resistant genetic information to other bacteria by horizontal gene transfer [2]. The aim of our work is the analysis of the bacterial community and the genes responsible for antibiotic resistance from samples obtained of plastic particles and raw water from rivers. All bacterial isolates were tested for their ability to form biofilm on two types of microplastic particles (PET, PS) by using fluorescence microscopy. Metagenomic sequencing by MinION was utilized to identify and characterize the abundance of ARGs present in a microbial community of the water. Obtained metagenomics reads were subjected to ARG annotation against the Comprehensive Antibiotic Resistance Database v 3.0.4 (CARD) using Diamond Blastx and the absolute abundance was calculated. The most abundant gene across the analyzed samples was *rpoB*, whose mutations have been associated with resistance to the antibiotic rifampicin, which is commonly used to treat tuberculosis and other bacterial infections. Additionally, the ARGs can be detected using qPCR assays oriented to diverse gene markers. Since we aim to obtain even the smallest amount of resistance genes in water, we used an even more sensitive method OSN-qPCR.

This study was mainly financed by the bilateral SAS (Slovak Academy of Sciences) – MOST (Taiwan) Joint Research Project (SAS-MOST/JRP/2020/1122/ PathogenTracker). This study has been also supported by the Slovak Academy of Sciences and the EIG CONCERT-Japan (EIG CONCERT-Japan/2019/881/ SuWaCer) and the European Regional Development Fund project: 313011V578.

[1] M. Amarasiri et al., Crit Rev Environ Sci Technol. 50(19), 2020

[2] K. Kaur et al., Chemosphere. 291, 2022

OP13

Antimicrobial Photodynamic Inactivation - A Promising Approach to Killing Infectious Pathogens

Helena Bujdáková[1], Matúš Štefánek[1], Katarína Bilská[1], Jarmila Czucz Varga[1], Nitin Chandra teja Dadi[1,2]

[1]Comenius University in Bratislava, Faculty of Natural Sciences, Department of Microbiology and Virology, Bratislava, Slovakia
[2]Department of Surgery, McGill University, Montreal, Canada

helena.bujdakova@uniba.sk

Healthcare-associated infections caused by yeasts of *Candida albicans* and *Candida auris*, and methicillin-resistant *Staphylococcus aureus* (MRSA) are a serious challenge because of resistance and ability of those microbes to form single or mixed biofilms on medical devices. Therefore, searching for alternative ways, how to eradicate problematic microorganisms and their biofilms is a current issue. While photodynamic therapy has been in the use for many years, antimicrobial photodynamic inactivation (aPDI) is only at the beginning of its practical application in medicine.

aPDI utilizes the photosensitizer (PS)-mediated and light-induced formation of reactive oxygen species (ROS) to kill microorganisms. Our research presents the action of aPDI using two PS, namely methylene blue or phloxine B, to biofilms formed by *C. albicans*, *C. auris*, and MRSA strains. Additionally, it provides an analysis of the mode of action as well as the participation of efflux genes involved in resistance to conventional drugs.

For this purpose, different approaches have been involved: determination of viability by colony forming unit calculation, measurement of ROS generation by chemiluminescence, estimation of change in efflux gene regulation using RT-q PCR, and various microscopies (CLSM, SEM).

aPDI efficiently reduced the survival of biofilms formed by all microorganisms with respect to tested PS, concentration, and duration of irradiation. Generally, the reduction of single biofilms showed a 10 to over 1000-fold reduction in growth, while mixed biofilm of *C. albicans*/*S. aureus* manifested lower susceptibility (10 to 100 times). Phloxine B showed excellent aPDI against MRSA biofilm, including that formed on polyurethane nanocomposite with a hybrid film based on clay mineral saponite functionalized with PS. A significant generation of ROS was observed after irradiation. Microscopy revealed an increased number of dead cells after aPDI (CLSM) and cell disruptions (SEM). All resistant microorganisms manifested overregulation of the efflux genes coding for Cdr proteins (*C. albicans* and *C. auris*)

or MFS (the MDR1 and *norA* genes for *C. albicans*, *C. auris*, and *S. aureus*, respectively).
Despite the activity of efflux transporters, these did not significantly affect aPDI, which proved to have great potential for the eradication of resistant biofilms.

Acknowledgement. This work was supported by the Slovak Research and Development Agency under contracts of APVV-18-0075 and APVV-21-0302.

OP14

Microbiome Patterns and Biomarker Discovery in *Clostridioides difficile* Infection: Insights from Human Cohorts and *in vitro* Models

Aleksander Mahnic[1], Maja Rupnik[1,2]

[1]*National Laboratory of Health, Environment and Food, Slovenia*
[2]*Faculty of Medicine, University in Maribor, Slovenia*

aleksander.mahnic@nlzoh.si

Clostridioides difficile infection (CDI) is a major healthcare-associated infection that causes a range of clinical manifestations, from mild diarrhea to severe pseudomembranous colitis and toxic megacolon and is strongly influenced by the interactions between the pathogen and the host's microbiome. In the Department of Microbiological Research at the National Laboratory of Health, Environment, and Food, we aim to investigate interactions between *C. difficile* and gut microbiota from various perspectives, including population and in vitro studies.
Data from a study on a cohort of CDI-positive and CDI-negative patients hospitalized in the gastroenterology department at the University Clinical Centre in Maribor shows that patients with concomitant CDI and inflammatory bowel disease exhibit exacerbated disruption in the microbiota, indirectly explaining more severe CDI reported in IBD patients in other studies. Additionally, findings from simple *in vitro* gut models demonstrate the effect of microbiota on *C. difficile* and ribotype-dependent microbiota modulation by *C. difficile*. Moreover, we have identified a *C. difficile* antagonist, namely *Clostridium sporogenes*, whose secondary metabolite inactivates both *C. difficile* toxins, TcdA and TcdB. Our findings provide new insights into the interactions between the pathogen and the host's microbiome and open up new avenues for the development of targeted therapies to combat CDI.

OP15

Structure-Activity Relationships of *Trichoderma peptaibols* - A Crucial Step Towards Practical Applications

Dóra Balázs, Tamás Marik, Ákos Rozsnyói, András Szekeres, Csaba Vágvölgyi, Chetna Tyagi, László Kredics

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary
dora.balazs91@gmail.com

The species from the genus *Trichoderma* gain importance in agriculture and biotechnology due to their produced secondary metabolites, among which peptaibols are of particular importance. *Peptaibols* are characterized by a high degree of amino acid variability in their sequences due to their synthesis carried out by non-ribosomal peptide synthetases (NRPSs) with modular structure. Peptaibol extracts are able to inhibit Gram-positive bacterial and several fungal species, thereby, based on earlier experiments, peptaibols may have the potential to support plant growth and provide protection against plant pathogenic microorganisms. For a comprehensive examination, in addition to laboratory experiments, modern computational modeling techniques, such as accelerated molecular dynamics (aMD) simulations can provide a deeper understanding of the mechanism of peptaibol action.
During our experiments, peptaibol production of 8 *Trichoderma* strains were determined and the purified peptaibol extracts tested against commonly known 11 Gram-positive and Gram-negative bacterial strains as well as two plant pathogenic fungal species. The minimum inhibitory concentration (MIC, mg ml^{-1}) and effective concentration (EC, mg ml^{-1}) values of the purified peptaibol extracts were determined in laboratory tests. In order to understand the action mechanism of peptaibols, *in silico* aMD simulations were carried out to uncover the folding process of the compounds with different sequences. To understand the structure-activity relationships (SARs) of peptaibols, the sequences were modelled using the aMD simulation technique and compared with the results of the MIC and EC tests, to be able to correlate folded peptaibol dynamics affected by the amino-acid content and sequence length with their expressed bioactivity.
The results of SARs improve the knowledge of correlational relationships between conformation and bioactivity, may lead to an effective design of peptaibiotic intervention required for plant disease management and can also facilitate the potential future application of peptaibols in agriculture.

This research was supported by the National Talent Programme NTP-NFTÖ-22-B-0099 (Hungary).

OP16

Whole Genome Sequencing and Machine Learning Assisted Study to Understand Diffusion Dynamics of *Legionella pneumophila* in a Local Area

Andrea Colautti[1], Andrea Brunello[2], Nicola Saccomanno[2], Lucilla Iacumin[1], Antonella Felice[3], Marinella Franchi[3], Marcello Civilini[1]

[1]*Department of Agricultural, Food, Environmental and Animal Science (Di4A), University of Udine, Udine, Italy*
[2]*Department of Mathematics, Computer Science and Physics, University of Udine, Udine, Italy*
[3]*Laboratory of Microbiology, ARPA – Regional Agency for Environmental Protection Friuli Venezia Giulia, Udine, Italy*

andrea.colautti@uniud.it

Legionella spp. are considered a significant cause of potentially preventable morbidity and mortality. Given the increasing number of legionellosis cases, particularly those caused by *L. pneumophila* serogroup 1, developing monitoring techniques to prevent infections caused by this pathogen is becoming increasingly important. In this study, the main factors responsible for the spread of *L. pneumophila* ser. 1 and 2-15 in a defined geographical region were determined using a machine learning approach on 23,837 water samples, and the stratification based on isolation location was then validated at a genetic level by sequencing 200 representative strains. In this regard, an unsupervised spatiotemporal clustering analysis was performed using the SaTScan software. A model with a supervised methodology was built using XGBoost to analyze the contribution of other features (day, longitude, latitude, building category, and weather conditions) to the presence of *L. pneumophila*. For the genetic analysis, the genomes after being sequenced using Illumina MiSeq platform were assembled using the WGA-LP pipeline and were analyzed with Roary, pyMLST, Phylonium, and PhyloPhlAn to obtain clusters based on different genetic features. Following these analyses, it was possible to observe the prediction in defined areas (<5 km) of 7 spatiotemporal clusters with unusually high contamination levels by the unsupervised learning model, with results consistent with previous studies. From the supervised learning results instead, it was possible to obtain predictions both at the level of contamination and of serogroup. In both cases, the most important variables were the geographical ones and the category of isolation building. In particular, health facilities buildings were found to be the most correlated to higher contaminations and the presence of *L. pneumophila* ser. 1. The genetic analysis showed consistent phylogenetic patterns from all the clustering methods, identifying eight main clusters. However, no correlations were found between the

period or area of isolation. Instead, in accordance with what was observed with the supervised learning analyses, a strong correlation was observed between the clusters and the type of category of building. The findings, consistent with literature research, confirm that health facilities are the most vulnerable, both in terms of contamination and the presence of specific strains of *L. pneumophila* ser. 1.

POSTER PRESENTATIONS

P1

Study of Microorganisms Involved in N Cycle from Technogenic Soil Affected by Soda Industry

Sweta Binod Kumar[1], Agnieszka Kalwasińska[1], Piotr Hulisz[2], Maria Swiontek Brzezinska[1], Agnieszka Piernik[3]

[1]Department of Environmental Microbiology and Biotechnology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Lwowska, Toruń, Poland
[2]Department of Soil Science and Landscape Management, Faculty of Earth Sciences and Spatial Management, Nicolaus Copernicus University in Toruń, Lwowska, Toruń, Poland
[3]Department of Geobotany and Landscape Planning, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Lwowska, Toruń, Poland

kala@umk.pl

Anthropogenic activities have huge impact on landscapes that can change the structure and properties of soil such as industrialized zones having high risk of soil salinization. Such soils established due to anthropogenic activities are known as Technosoils. One of those disturbed soils which is heavily affected by soda industry is located in the proximity of soda lime repository ponds of CIECH Soda Polska S.A (Inowrocław, central Poland). The microbiome plays a crucial role in the biological functioning of degraded lands. However, the presence of specific guilds of microorganisms involved in the N cycle, has not been thoroughly studied. Also, microorganisms retrieved from such habitats are naturally adapted to extreme environment that can be used for isolating bacteria useful for agriculture e. g. salt-tolerant PGPR. Therefore, the present work aims to address this knowledge gap by assessing the microbiota responsible for N cycle in relation to soil properties and plant species composition across two transects reflecting different land use types (saline wasteland and arable fields) and isolation of salt-tolerant diazotrophs with plant growth-promoting potential from the rhizosphere of plants growing on the same. The studied soils were alkaline (pH-H2O 7.4-8.0) and ranged from non-saline to strongly saline (ECe 1.0-58.1 dS m^{-1}). Our findings showed that high soil salinity reduced the abundance of nitrifying microorganisms, while denitrifying bacteria and N_2-fixers were more resistant. Moreover, high soil pH had positive correlation with bacterial *amoA* gene copy numbers. Furthermore, varied cultivable N_2-fixers were isolated from the technosoils with various PGPR properties. Isolates Azo 12 and W4 ii identified as *Agrobacterium* sp. and *Azotobacter chroococcum* respectively, significantly ($p < 0.001$) promoted the growth of seedlings' roots and leaves in the saline conditions (150 mM) compared to seedlings without bacteria. Interestingly, other isolates related to *Agrobacterium* sp. (Azo 12), *Sphingobium fuliginis* (Azo 7) and

Variovorax paradoxus (Azo 11) significantly ($p < 0.05$) increased seedlings' growth in nitrogen stress compared to control seedlings. The study can be valuable for land reclamation projects and revealed that technosoils developed by soda lime industry are the unexplored source of various cultivable salt-tolerant PGPR. The present work is a part of project NitroFixSal, Project code: 101038072, funded under H2020 | MSCA-IF-EF-ST.

P2

Formation of Synthetic Bacterial Consortia Through Electrostatic Manipulation of Microbial Cells

Aleš Lapanje[1], Tomaž Rijavec[1], Dmitrii Deev[1, 2], Maja Zugan[1, 2], Anatolii A. Abalymov[1]

[1]*Jozef Stefan Institute, Slovenia*
[2]*Jozef Stefan International Postgraduate School, Slovenia*

ales.lapanje@ijs.si

Among four fundamental physical forces, only electromagnetism through electrostatics can be applied to biological objects. From the electrostatic point of view under normal physiological conditions microbial cells resemble negative surface potential that is attributed to the negative chemical groups present in the molecules, which are integral components of the membrane and cell wall. A stronger charge increases repulsive forces among the cells and in order to attach cells to each other this barrier must be overcome. Therefore, in our laboratories, we were focused on the development of a set of different approaches based on the electrostatic interactions that enable the physical attachment of alive bacterial cells to other cells or on different inanimate surfaces. Using the approach and manipulation of conditions (e.g., ionic strength, flow, the density of cells, etc.) we were able to construct different synthetic polymicrobial structures such as biofilms and aggregates where we were able not only to put cells together but also spatially distribute them by the design. Through studies of the formation and further development of the structures in time, we obtained insights on (i) the self-organisation of structures where strict anaerobes and aerobes became associated, (ii) observing niche separations by the formation of anaerobic and aerobic zones and (iii) rewiring ecological interactions from competition to the cooperation. In relation to that, we were able to determine the intensity of formation of the most toxic Hg species, the Met-Hg, in aerobic conditions if appropriate interspecies interactions can occur. In addition, we also showed that the obtained basic knowledge can be also very well translated to the bioremediation applications such as precipitation of toxic metals such as Zn, Pb, and U in polluted waters as well as degradation of organically polluted waters and soils by designing smart carriers which were then used in bioaugmentation strategies.

P3

Organomercurial Lyase (MerB) Enabled Methylmercury Detection

A. F. P. Allwin Mabes Raj[1, 2, 3], Tomaz Rijavec[1], Milena Horvat[1], Aleksandra Lobnik[3, 4], Aljoša Košak[3, 4], Aleš Lapanje[1]

[1]*Department of Environmental Sciences, Josef Stefan Institute, Ljubljana, Slovenia*
[2]*Jožef Stefan International Postgraduate School, Ljubljana, Slovenia*
[3]*IOS, Institute of Environmental Protection and Sensors, Ltd., Maribor, Slovenia*
[4]*University of Maribor, Faculty of Mechanical Engineering, Centre of Sensor Technology, Maribor, Slovenia*

ales.lapanje@ijs.si

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 catalyzes 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+}) [1]. A MerBADtt complex consisting of the MerB, a mercuric ion, a dithiothreitol (DTT) and mercuric reductase MerA is then releasing Hg^0 (elemental mercury) [2]. Since MerB is a highly specific enzyme, we are planning to use its Met-Hg-specific binding characteristics as a sensing component of the sensor. The formed complex of MerBADtt can act as a transducer if combined with precise Hg^0 measurement. In order to achieve that, we have prepared an expression system that will enable us to obtain a high enough amount of MerB and MerA enzymes. The expressed MerB and MerA enzymes with his-tag were purified and evaluated for their use in the preparation of the Met-Hg-specific sensor. The obtained MerB activity was determined through MeHg binding and conversion to Hg^{2+} using cold vapour atomic fluorescence spectroscopy (CVAFS). The further reduction of Hg^{2+} by the whole complex was measured using the Lumex mercury analyzer. In order to use this complex in sensing applications, we determined the sensitivity, durability and specificity of this approach in real time using various environmental matrices.

P4

Exploiting the Potential of Marine Microbes as Indicators of Anthropogenic Disturbance in Coastal Marine Areas

Ana Ramljak[1], Ivana Babić[1], Jurica Žučko[2], Martina Furdek Turk[1], Dimitrios Karpouzas[3], Sotirios Vasileiadis[3], Mavro Lučić[1], Slavica Matijević[4], Maja Fafanđel[5], Nikolina Udiković Kolić[1], Ines Petrić[1]

[1]*Ruđer Bošković Institute, Zagreb, Croatia*
[2]*Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia*
[3]*Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece*
[4]*Institute of Oceanography and Fisheries, Split, Croatia*
[5]*Center for Marine Research, Ruđer Bošković Institute, Rovinj, Croatia*

aramljak@irb.hr

Through monitoring and defining the environmental status of their marine waters under the Marine Strategy Framework Directive, Mediterranean countries are constantly striving to improve their marine protection policies. However, the MSFD doesn't consider microbial communities in its monitoring, although their role in the functioning of the marine ecosystem has already been scientifically proven. With the goal of highlighting the importance of marine microbial communities and defining them as potential indicators of anthropogenic disturbance, the MicroLink project focuses on marine sediment, which acts as a long-term contaminant sink. Sediment samples were collected from seven ports in the Adriatic Sea that had previously been flagged as highly polluted sites. Physicochemical parameters were measured, and the collected data were transformed using ilr (isometric log ratio) transformation. This was followed by calculation of balances what enabled a correct representation of the data in Euclidean space. Hierarchical clustering was used to classify the samples into 5 classes of anthropogenic disturbance (low, mild, medium, high and extreme). After DNA extraction, statistical analysis of the amplicon sequence data was performed. At the family level, there were no significant differences in structure and diversity among the different disturbance levels. NMDS analysis of the taxonomic data indicated clustering of samples on a regional basis (northern and southern Adriatic) rather than disturbance levels. However, families such as *Woeseiaceae* and *Rhodobacteriaceae* (bacteria), as well as *Chytridiomycetes* (fungi) and *Diatomea* (protists) showed an increase in abundance at sites identified as extreme. As the MicroLink project integrates different approaches to study the benthic microbial communities, the functional level still needs to be explored using shotgun metagenomic sequencing, which will certainly provide a deeper insight into the potential microbial indicators of anthropogenic disturbance.

P5

Effects of Intermittency on Microbial Communities, Biogeochemical and Ecological Process in IRES Ecosystem

Ines Petrić[1], Anamarija Kolda[2], Antonija Kulaš[3], Lucia Pole[4], Helena Senko[1], Marija Gligora Udovič[3]

[1]*Laboratory for Environmental Microbiology and Biotechnology, Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia*
[2]*Laboratory for Aquaculture and Pathology of Aquatic Organisms, Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia*
[3]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*
[4]*Laboratory for Biodiversity, Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia*

Anamarija.Kolda@irb.hr

Intermittent rivers and ephemeral streams (IRES) are ubiquitous river ecosystems (51-60% of rivers worldwide) that periodically stop flowing and/or dry up. They are typically affected by anthropogenic interventions, exacerbated by climate change, which further extends the duration of dry periods. In addition, many formerly perennial rivers are experiencing intermittence. Microorganisms are often overlooked in IRES oriented research, even though they are responsible for the stability and sustainability of river ecosystems. This research aims to provide data on changes in microbial community structure and functions as a result of fluctuations in hydrological phases, especially dry periods.

The Krčić River, a naturally occurring IRES in the Dinaric Karst region (Croatia), was selected as a "natural laboratory" to predict the behavior of the future river ecosystem affected by flow intermittence. Sampling of microbial mats was conducted from April 2019 to May 2020 (except March and April). Physicochemical parameters and nutrients were measured, and total DNA and RNA were extracted for amplicon sequencing of 16*S* rRNA and 18S rRNA genes, while selected functional genes (*nifH, amoA, narG, nirS, nirK, nosZ, rbcl, anammox* 16S rRNA, *sox, and dsrB*) were determined by qPCR.

The results obtained showed that *Alphaproteobacteria* (48%) and *Oxyphotobacteria* (35%) dominated the community of active bacteria (averaged over the whole year and all samples). Cyanobacteria were most active in the microbial mat in May, June, October 2019, and February, and May 2020 (50,4%). *Alphaproteobacteria* dominated the active community in April, June, July, August, November 2019, and January 2020 (67,7%). *Ochrophyta* were the most active members of the eukaryote community (73-92%). Dry season (October) samples had a distinct community consisting mainly of *Chlorophyta* (44%), *Ascomycota* and *Basidiomycota* (25%), and *Alveolata* (12%), with a high percentage of rare

taxa. Of the 12 functional genes analyzed by qPCR, 9 different genes involved in the N cycle (nitrification/oxidation of ammonia, denitrification, nitrogen fixation), carbon cycle (Calvin-Benson-Basham cycle, photosynthesis), and S cycle (sulfur oxidation) were detected.

The results suggest that microbial mat communities have good bioindicator potential in which adaptation and evolutionary processes related to climate change could be studied to enable effective protection of habitats such as IRES in the future.

P6

Will Predicted Increasing Flooding Frequency Alter Soil Microbial Communities and Functions?

Anastazija Huđ[1], Helena Senko[1], Ivana Babić[1], Jurica Žučko[2], Goran Palijan[3], Ellen Kandeler[4], Sven Marhan[4], Marko Petek[5], Nikolina Udiković Kolić[1], Ines Petrić[1]

[1]*Institute Ruđer Bošković, Zagreb, Croatia*
[2]*Faculty of Food Technology and Biotechnology, Zagreb, Croatia*
[3]*University of Osijek, Department of Biology, Osijek, Croatia*
[4]*University of Hohenheim, Stuttgart, Germany*
[5]*University of Zagreb, Faculty of Agriculture, Zagreb, Croatia*

ahudj@irb.hr

Floods, droughts, heat waves, cold waves – climate change is leaving visible traces and threatening ecosystems, energy security, food supply, water supply and infrastructure. As agriculture is directly dependent on weather conditions, it is one of the sectors most affected by climate change. As crop productivity and quality decline due to climate change, research and action are needed to develop new approaches to adapt to a rapidly changing climate. Soil microorganisms play a central role in nutrient transformation in agricultural soils by mediating organic matter decomposition and cycling of C, N, P, and S elements, and therefore should be strongly considered when discussing the impacts of climate change on agricultural production. The project "Potential of the rhizosphere microbiome in the adaptation of agriculture to climate change" (PERSPIRE, EU Regional Development Fund) investigates the effects of flash floods, which occur as a result of excessive rainfall, on the plant holobiont, focusing on the microbiome residing within the soil. The experiment was conducted under semi-controlled greenhouse conditions using cabbage (*Brassica oleracea* var. *capitata f. alba*) as a model plant and involved testing two scenarios: (i) two repeated long-term floods (7 days) and (i) one long-term flood on later growth stage plants. We followed the effects of flooding on the soil microbiome both at (i) structural level: changes in bacterial (16S rRNA) and fungal community (ITS2 gene sequencing) and at (ii) functional level: changes in abundance of N-cycle genes - *amoA*, *amoB*, *nirS*, *nirK*, *nosZ* - by qPCR and changes in five extracellular hydrolytic enzymes - spectrophotometrically. Long-term flooding was shown to induce (short-term) changes in the structure of both bacterial and fungal soil communities, revealing changes in the abundance of several subpopulations (*Creanarcheota*, *Acidobacteriota*, *Bacteroidota*, *Proteobacteria*, *Ascomycota*). In addition, qPCR results indicated an impact of flooding on soil nitrogen cycling, while enzyme activity gave insight into the rates of processes such as decomposition of organic matter and mineralization of nutrients. Because of the enormous importance of soil microorganisms in plant development, such disturbances at the level of the soil microbiome could have strong implications not only for soil health but also for plant health.

P7

Using Nano- And Microscale Vaterite Particles as a Platform for Studying the Effect of Recalcitrant Agents on Bacterial Consortia

Anatolii A. Abalymov, Dmitrii Deev, Aleš Lapanje

Department of Environmental Sciences, Jozef Stefan Institute, Ljubljana, Slovenia

anatolii.abalymov@gmail.com

Nanoparticles and microparticles of various materials have been studied as potential platforms for drug delivery, sensing, and imaging in biomedical applications. In recent years, they have also been explored for their potential use in studying the effects on degradation of recalcitrant substances. Both, nanoparticles and microparticles, can provide a platform for studying the local effects of recalcitrant substances on bacterial cells since their physicochemical properties can be precisely tuned and due to the porosive nature they can be loaded with specific chemical substances. The porous synthetic particles can act as a model system of the soil particles where the access to microbes as well as the release of recalcitrant pollutant can be studied. Moreover, by attachment of different strains capable of transformation of recalitrant and complex molecules such as PAH, it can be studied how metabolically compatible environmental strains are with the isolated strains in the bioremediation processes.

In this work, we aimed to construct cheap porous vaterite particles with pollutants adsorbed within the pores. As such the particles get slowly recristalized into calcite, squeezing out the pollutant. We used these particles as attractants for pollutant degraders or as microbes that can be involved in different steps of the mineralisation. We used two strategies (i) exposing particles to the extracted cells and (ii) exposing particles with attached cells that can partially degrade the pollutant. Synthesized vaterite particles with a diameter of 0.8, 3.5 and 11 μm were used since size determined the level of the surface interaction as well as loading capacity. The methods such as scanning electron and fluorescence microscopy, flow cytometry, etc. were used to characterize the particles, detect bacterial adsorption on the surface of the vaterite particles, and bacterial viability and behavior.

P8

Effect of QS Molecule Farnesol on Mixed Biofilms of *Candida albicans* and *Staphylococcus aureus*

Barbora Radochová[1], Samuel Kendra[1], Luisa Jordao[2], Laura Kursawe[3,4], Judith Kikhney[3,4], Annette Moter[3,4,5], Helena Bujdáková[1]

[1]*Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia*
[2]*Department of Environmental Health, Research and Development Unit, National Institute of Health Dr. Ricardo Jorge (INSA), Portugal*
[3]*Biofilmcenter, Institute of Microbiology, Infectious Diseases and Immunology, Charité—Universitätsmedizin Berlin, Germany*
[4]*MoKi Analytics GmbH, Charité-Universitätsmedizin Berlin, Germany*
[5]*Moter Diagnostics, Berlin, Germany*

barbora.gaalova@uniba.sk

Signaling molecules have been intensively studied in recent years due to their potential to control pathogens and appear to be ideal candidates for the development of combination therapy. Farnesol (FAR) synthesized by *C. albicans* in a concentration-dependent manner plays a central role in biofilm physiology. In this work, the effect of FAR alone and in combination with antibiotics was studied on planktonic bacteria *S. aureus* (MSSA and MRSA), as well as on mixed biofilms of *C. albicans* and *S. aureus*. In planktonic bacteria, a synergistic effect of FAR with beta-lactams, a moderate effect with kanamycin and no effect with ciprofloxacin was observed. For this experiment, E-tests were used in the presence of two concentrations of FAR (150 μM and 300 μM). The antibiofilm potential of FAR (62.5 – 1 000 μM) was measured by the XTT method in terms of MBIC50. The MBIC50 of FAR for mixed biofilms of *C. albicans*-MSSA1 and *C. albicans*-MRSA2 was established as 125 and 250 μM, respectively. The combination of FAR (300 μM) with OXA (2 mg/mL) tested on mixed biofilms resulted in 80% inhibition compared to 4% inhibition after treatment with the same concentration of OXA alone. Scanning electron microscopy was used to characterize the architecture of biofilms and the impact of the studied antimicrobial agents on biofilms. Significantly less candidal hyphae were observed in the samples treated with FAR (300 μM) and FAR (300 μM/OXA 2 mg/mL). However, only a slight difference was observed between biofilm treated with FAR and a combination of FAR/OXA using microscopy as well as XTT method. Therefore, FISH was applied as a tool for monitoring the activity of the microbial cells within biofilms based on ribosome content. Candidal cells were active in all samples of mixed biofilms, whereas bacteria were partially active only in the FAR-treated sample and no activity was observed in the sample treated with FAR/OXA.

This suggests a synergistic effect of FAR that may sensitize these cells to OXA. We may conclude that FAR acts on several levels. By blocking hyphae, it prevents the formation of a compact biofilm and, at the same time, increases the sensitivity of MSSA or MRSA to beta-lactam antibiotics.

This research was supported by Slovak Grant Agency under the contract no. APVV-18-0075, APVV-21-0302 and SK-PT-18-0006. This work was also supported by the EU Grant number 952398—CEMBO.

P9

Newly Isolated Microbial Species with Keratinolytic Activity Show Promising Potential for Environmental and Industrial Applications

Blaž Petek, Romana Marinšek Logar

Biotechnical faculty, University of Ljubljana, Department of Microbiology, Slovenia

blaz.petek@bf.uni-lj.si

Enormous amounts of various organic wastes and by-products are generated annually. Wastes from slaughterhouses, meat and poultry plants and the wool textile industry contain many fibrous proteins such as collagen, elastin, and keratin. Keratinolytic microorganisms and their enzymes can play an important role in degrading keratin-rich wastes from various industries through environmentally friendly processes. There are many studies focusing on the degradation of chicken feathers, but there is a lack of highly active keratinolytic microorganisms capable of degrading the hard structure of wool fibres. Therefore, the aim of our study was to isolate new highly keratinolytic microbial strains that could be further used in biotechnological approaches for waste sheep wool processing, such as wool composting and biogas production, or even for keratinase production due to their wide use in different industries. In this paper, we present only part of the results of our study focusing on the highly active bacterial isolates. The microorganisms were isolated from the soil under the sheep wool pile. The most active isolates were identified by 16S rRNA sequencing followed by whole genome sequencing. Characterisation of the keratinases included determination of pH and T optimum, the molecular weight of the enzymes, and the effect of various chemicals on enzyme activity. The identification revealed that the isolates belong to 4 different *Bacillus* species (*B. subtilis, B. mycoides, B. wiedmannii, B. altitudinis*). The keratinolytic activity of *B. mycoides* and *B. wiedmannii*, both belonging to the larger *B. cereus* group, has never been reported before. Both keratinases were active over a wide range of pH and T, with an optimum at pH 8 and 55 °C. The molecular weight of the keratinases was determined to be ~35 kDa. Molecular weights of known keratinases range from 18-240 kDa, while a 39 kDa keratinase was previously found in *B. altutudinis*, which also belongs to the *B. cereus* group. With their wide pH and temperature range, the new isolates offer promising prospects for use as starter cultures for waste sheep wool waste composting (bioaugmentation) and microbial or enzymatic pretreatment of waste sheep wool for biogas production. The wide temperature range is particularly advantageous for composting, as the keratinolytic enzymes can be active at all stages of composting from mesophilic to thermophilic (up to 70 °C), which is very important for the hygienization of the substrate.

P10

Chlorine Disinfection Modifies the Antibiotic Resistant Population of Hospital Wastewater – A Nanopore Long-Read Metagenomic Approach

Damian Rolbiecki[1], Monika Harnisz[1], Łukasz Paukszto[2], Jakub Sawicki[2]

[1]*Department of Water Protection Engineering and Environmental Microbiology, Faculty of Geoengineering, University of Warmia and Mazury in Olsztyn, Poland*
[2]*Department of Botany and Nature Protection, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland*

damian.rolbiecki@uwm.edu.pl

Hospitals are hotspots for highly antibiotic-resistant bacteria (ARBs). One way for ARBs to spread outside the hospital environment is through hospital wastewater (HWW). To reduce that risk, HWW is often treated in local disinfection station. Chlorine-based wastewater disinfection is widely used around the world. Recent studies suggest that, apart from its main function, HWW disinfection with the use of chlorine compounds can also significantly affect the spread of drug resistance.
The aim of this study was to analyze changes in the HWW microbiome caused by the process of chemical disinfection based on chlorine compounds. The study focused on the analysis of changes in the drug-resistant population (ARB population). The nanopore method was used to sequence the metagenome DNA of HWW samples collected before and after the disinfection process in 4 research seasons. The long reads obtained enabled the direct identification of the host taxa of ARGs.
The results indicate that the ARB population was taxonomically different from the total bacterial community in the HWW and it was only a fraction of the total bacterial population present in the HWW. Significant differences were found in the ARB population between the sites studied. In the tested samples, antibiotic resistance genes were most often identified in bacteria belonging to the classes *Gammaproteobacteria*, *Bacteroidia* and *Bacilli*. In the wastewater after the disinfection process, more unique hosts of ARGs were detected than in the wastewater before disinfection. Disinfected wastewater also showed significantly higher ARB population diversity. Disinfection influenced changes in the structure of drug-resistant critical alarm pathogens present in HWW. Changes in their species composition, encoded drug resistance determinants and the frequency of their detection were observed.
The research provides new insights into the changes in the population of drug-resistant bacteria present in HWW. The results suggest that disinfection with chlorine compounds significantly modifies the ARB community and may contribute to the transfer of drug resistance determinants to new, previously drug susceptible, bacterial taxa. Consequently, chlorine-based disinfection practices may pose a risk to the environment and public health by accelerating the spread of antimicrobial resistance.

P11

SSB Protein Preserves Genome Stability in *Escherichia coli*

Edyta Đermić[1], Davor Zahradka[2], Isidoro Feliciello[3], Siniša Ivanković[4], Damir Đermić[2]

[1]*Division of Phytomedicine, Faculty of Agriculture University of Zagreb, Zagreb, Croatia*
[2]*Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*
[3]*Dipartimento di Medicina Clinica e Chirurgia, Universita' degli Studi di Napoli Federico II, Napoli, Italy*
[4]*Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*

dermic@irb.hr

Preserving genome stability is of critical importance to living organisms. Lack of genome stability results in severe conditions such as cancer and premature aging in mammals. Stability of *E. coli* genome is determined by metabolism of 3´-ending tails at DNA double-strand breaks, which are faithfully mended by homologous recombination catalyzed by RecBCD enzyme. The rare cases of aberrant DNA transactions that lead to imprecise recombination events in wild-type *E. coli* are unwound by prototypic genome guardian, RecQ helicase, thus preventing the appearance of illegitimate recombination. We used ssb-1 conditional mutation and measured the frequency of aberrant λ-prophage excision from the *E. coli* genome (Spi- assay) for quantifying illegitimate recombination in the bacteria of wild-type as well as of several other *E. coli* genetic backgrounds. We reveal that SSB protein prevents illegitimate recombination, which depends on SSB binding to ssDNA. Since for illegitimate recombination SSB overproduction complements RecQ deficiency, our results suggest that SSB prevents appearance of aberrant recombination events, whereas RecQ dismantles those that do occur in the *E. coli* genome.

P12

Chromosome Segregation and Cell Division Defects in *Escherichia coli* After Induction of Double-Strand DNA Breaks

Ksenija Zahradka, Jelena Repar, Damir Đermić, Davor Zahradka

Laboratory for Molecular Microbiology, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

zahradka@irb.hr

In *Escherichia coli*, double-strand DNA breaks (DSBs) are repaired by the RecBCD pathway of homologous recombination. In the initiation (presynaptic) stage of this process, the RecBCD enzyme resects double-strand DNA ends and loads RecA recombinase to the emerging single-strand DNA tails. RecA catalyzes the central reactions of homologous DNA pairing and strand exchange (synapsis), while RuvABC complex and RecG helicase process recombination intermediates (post-synapsis). In this work we have studied chromosome morphology and segregation after introduction of DSBs by I-SceI endonuclease in *E. coli* strains carrying mutations in presynaptic (*recB, recO*) and postsynaptic (*ruvABC, recG*) recombination functions. Induction of DSBs by I-SceI caused severe chromosome segregation defects and DNA-less cell formation in the *ruvABC, recG*, and *ruvABC recG* mutants. This phenotype was efficiently suppressed by the *recB* mutation, while the *recO* mutation had no suppressive effect. These findings indicate that attempted DSB repair via the RecBCD recombination pathway influences DNA distribution within the cell as well as its transmission upon cell division. We assume that the DNA strand exchange between sister chromosomes during homologous recombination causes a temporary delay in chromosome segregation and disruption of cell division. In the absence of the postsynaptic RuvABC and/or RecG functions, the RecBCD-mediated DSB repair leads to accumulation of recombination intermediates that interfere with chromosome partition and cell division.

P13

Plasma for Microbes: How the Flame Could Help to Increase Biomass

Dmitrii Deev[1,2], Mark Zver[2,3], Nina Recek[3], Tomaž Rijavec[2], Gregor Primc[3], Aleš Lapanje[2]

[1]*Jožef Stefan International Postgraduate School, Ljubljana, Slovenia*
[2]*Department of environmental sciences, Jožef Stefan Institute, Ljubljana, Slovenia*
[3]*Department of Surface Engineering, Jožef Stefan Institute, Ljubljana, Slovenia*

dmitrii.deev@ijs.si

Modern society is faced with an emerging demand for green waste-water cleaning technologies. The most optimal activity of the bacterial biomass involved in the mineralization of pollutants is a crucial factor for the fast and efficient removal of many hazardous substances. Therefore, new methods for improving biomass growth are being extensively studied. Since a variety of substances are present in polluted water, the most efficient degradation might occur in the most diverse environment that can be provided in the fluidized bed bioreactors where cells get attached to suspended carriers. In order to get cells efficiently attached, the hydrophobicity as well as the negative charge of most of the surfaces of the carriers must be modified. New solutions to overcome these barriers as well as can be also easily upscaled could sufficiently improve biomass gaining on the carriers.

Hence, here we show a new approach for the surface modification of polyurethane chips that can be used as carriers in the fluid bed reactor waste-water treatment systems. We developed a procedure that enhances hydrophilicity and hypothetically makes it more favorable for the colonization of native wastewater microbiota. The biochips surfaces were treated with plasma in two different regimes, after which it has been populated with fluorescently pre-stained bacteria isolated from the wastewater power plant. Exposure of the modified surfaces was performed for 12 hours in a specially designed chamber and the attachment was evaluated using the fluorescent microscopy approach.

At the initial time point we observed 13 to 15 times increased attachment of native microbiota on plasma-treated in E and H regimes, respectively, then it was observed on the control surfaces. After prolonged incubation up to 12 hours of cultivation, this parameter increased by 31.68% for the chip treated in E-mode. In contrast, the H-mode treated surfaces as well as the control showed a 55% and 69% decrease in observable attached biomass, respectively.

The treatment of the surface by using plasma under nitrogen gas resulted in stimulated attachment of native microbes. The activity of attached microbiota and selectivity of the modified surfaces on the attachment of particular species are also further investigated since this determines the succession of the attached community as well as the longevity of the metabolic process involved in the degradation of the organic pollutants.

P14

Can Cyanobacteria and Microalgae Fractionate Tritium Isotopes

Farzaneh Hosseini[1,2], Maja Zugan[1,2], Dmitrii Deev[1,2], Marko Štrok[1,2], Tomaž Rijavec[2], Aleš Lapanje[2]

[1]*Jožef Stefan International Postgraduate School, Ljubljana, Slovenia*
[2]*Jožef Stefan Institute, Ljubljana, Slovenia*

Farzaneh.Hosseini@ijs.si

Tritium is one of the radioactive isotopes of hydrogen. Although it is a low-level beta-emitter (E_{max} = 18.6 KeV), it is very mobile in the environment passing from the environment and entering the food chain through plants. Therefore, it can pose a threat to human health if ingested at higher amounts. The most of the tritium is released to the environment during routine operation of nuclear power plants (NPPs), in nuclear fuel reprocessing plants, during nuclear accident and bomb testings. Although tritium is produced during these activities, the amount of it is at a below threshold level that can have some value, for example as fuel in the fusion reactors. Accordingly strategies are used to treat waste waters from nuclear power plants. Various physicochemical methods have been proposed for tritium removal. In this work we explored the potential of the fractionation of the tritium-contained water by photosynthetic activities. We tested this hypothesis by using cyanobacteria *Synechococcus elongatus* and *Synechococcus leopoliensis*, and microalgae *Chlorella sorokiniana*. According to our results we were able to enrich tritium approximately to the 20% from the initial concentration in the most optimal conditions. This simple and cheap approach can be used in initial stages for production of the fusion reaction fuels.

P15

Inter-Kingdom Microbial Interactions Revealed by a Comparative Machine-Learning Guided Multi-Omics Analysis of Industrial-Scale Biogas Plants

Roland Wirth[1,2], Zoltán Bagi[2], Prateek Shetty[1], Márk Szuhaj[2], Sally Cheung[2], Kornél L. Kovács[1,2], Gergely Marótia[1,3]

[1]*Institute of Plant Biology, Biological Research Centre, Szeged, Hungary*
[2]*Department of Biotechnology, University of Szeged, Szeged, Hungary*
[3]*Faculty of Water Sciences, University of Public Service, Baja, Hungary*

maroti.gergely@brc.hu

Multi-omics analysis is a powerful tool for the detection and study of inter-kingdom interactions, such as those between bacterial and archaeal members of complex biogas-producing microbial communities. In the present study, the microbiomes of three industrial-scale biogas digesters, each fed with different substrates, were analysed using a machine-learning guided genome-centric metagenomics framework complemented with metatranscriptome data. This data permitted us to elucidate the relationship between abundant core methanogenic communities and their syntrophic bacterial partners. In total, we detected 297 high-quality, non-redundant metagenome-assembled genomes (nrMAGs). The analysis of the 16S rRNA gene profiles of the nrMAGs indicated that the Firmicutes phylum exhibited the highest copy number, whereas the Archaeal domain had the lowest. Subsequent examination of the three anaerobic microbial communities revealed distinct temporal variations that were unique to each industrial-scale biogas plant. The relative abundance of various microbes as revealed by metagenome data were independent from corresponding metatranscriptome activity data. Interestingly, Archaea showed considerably higher activity than was expected from their abundance. Our investigation identified 53 nrMAGs that were present in all three biogas plant microbiomes, albeit with varying levels of abundance. The core microbiome exhibited a significant correlation with the principal chemical fermentation parameters, and no single parameter was found to have a dominant influence on the community composition. Various interspecies H2/electron transfer mechanisms were assigned to hydrogenotrophic methanogens in the biogas plants that ran on agricultural biomass and wastewater. Analysis of metatranscriptome data revealed that methanogenesis pathways were the most active of all main metabolic pathways. These findings highlight the importance of a combinatorial omics data framework to identify and characterise the activity of specific microbes in complex environments.

P16
Sound and Rhythm Impact on Soil Biofilm-Forming Ability

Karlo Fičko, Goran Palijan

Department of Biology, University of Osijek, Croatia

gpalijan@biologija.unios.hr

Present day civilization is producing alarming levels of noise which often is developed in soil proximity having an opportunity to change functioning of soil microorganisms. Yet, its effects on soil microbial communities are unexplored. To investigate the effects of noise, of different sound types we tested classical music of Mozart and Beethoven, construction, and road works noise and rhythm played on drums at the environmentally relevant sound levels and time intervals. All tested sounds impacted soil biofilm forming ability but differently between soil and land use types. Agricultural fields were less impacted by noise compared to forest soils. Interestingly only regular rhythm stimulated all tested samples. Such results are the first evidence that noise can change the functional properties of soil microbial communities and that its effects are dependent on the type of sound and rhythm.

P17

Single and Repeated Short-Term Waterlogging Results in a Small but Significant Response of Soil Enzymes

Goran Palijan[1], Helena Senko[2], Anastazija Huđ[2], Lucia Pole[2], Boris Lazarević[3], Marko Petek[3], Dunja Šamec[4], Lidija Brkljačić[2], Ivana Rajnović[3], Armin Mešić[2], Nikolina Udiković Kolić[2], Ines Petrić[2]

[1]*Department of Biology, University of Osijek, Croatia*
[2]*Institute Ruđer Bošković, Croatia*
[3]*Faculty of Agriculture, University of Zagreb, Croatia*
[4]*Department of Food Technology, University North, Croatia*

gpalijan@biologija.unios.hr

Soil enzymes are crucial in soil nutrient cycling providing a basis for soil fertility. Different environmental factors could be detrimental to enzymatic activity and one such factor, soil flooding, is rarely investigated although the increased frequency of floods by climate change would add to its importance. This issue was addressed within the project "Potential of the rhizosphere microbiome in the adaptation of agriculture to climate change (PERSPIRE)", funded by the EU Regional Development Fund. Effects of waterlogging on the functioning of soil microorganisms was assessed by conducing experiment in the controlled plant-growth chamber (16 hours day/8 hours night; 25 ˚C per day/20 ˚C per night; 60-70% relative humidity) using cabbage (*Brassica oleracea* var. *capitata f. alba*) as a model plant. In the experiment cabbage plants and its soil were exposed to either one (72 h duration) or two short-term floods (72 h duration, 10 days recovery between floods). At different time points (day 0, after flooding, and after the recovery period), all of the soil was removed from the pots, thoroughly mixed, and subsamples were collected for further analyses. In this study, we investigated the effects of waterlogging on the activity of beta-glucosidase, aril-sulphatase, glucosaminidase, and glycine-aminopeptidase, of hydrolytic activity measured by fluoresceine diacetate test, dehydrogenase activity and soil biofilm-forming ability. Effects of flooding in multivariate space were assessed by the ANOSIM test which revealed mild (global R: 0.104) negative effects on enzyme activity though statistically significant ($p < 0.006$). The interplay of flood and other environmental factors (e.g. drought) should be investigated in the future in more detail to study the effects of climate change also from the waterlogging side of the drying-rewetting cycle.

P18

Tho2 Moonlights in the mRNP Quality Control

Igor Stuparević[1], Valentin Beauvais[2], Bojan Žunar[1], Ana Novačić[1], Christine Mosrin-Huaman[2], A. Rachid Rahmouni[2,†]

[1]*Laboratory of Biochemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*
[2]*Centre de Biophysique Moléculaire, UPR 4301 du CNRS, Orléans, France*
[†]*Deceased April 2021*

istuparevic@pbf.hr

The eukaryotic THO complex coordinates the assembly of so-called messenger-ribonucleoprotein particles (mRNPs), a process that involves co-transcriptional coating of nascent RNAs with proteins. Once formed, mRNPs undergo a quality control step that marks them for either active transport to the cytoplasm or Rrp6/RNA exosome-mediated degradation in the nucleus. However, the mechanism behind the quality control of nascent mRNPs is still unclear. We investigated the co-transcriptional quality control of mRNPs in budding yeast by expressing the bacterial Rho helicase, which globally perturbs yeast mRNP formation. We examined the genome-wide binding profiles of the THO complex subunits Tho2, Thp2, Hpr1, and Mft1 upon perturbation of the mRNP biogenesis, and found that Tho2 plays two roles. In addition to its function as a subunit of the THO complex, free Tho2 targets Rrp6 to chromatin via its C-terminal domain. This finding raises the possibility that Tho2 is involved in co-transcriptional mRNP quality control independently of other THO subunits. Considering that both the THO complex and the RNA exosome are evolutionarily highly conserved, our findings are likely relevant for mRNP surveillance in mammals.

P19

The Effect of Viability on CRISPR-Cas Adaptation in *Escherichia coli*

Marin Radovčić[1,2], Ivana Ivančić Baće[1]

[1]*Faculty of Science, University of Zagreb, Croatia*
[2]*Xellia Pharmaceuticals, Zagreb, Croatia*

ivana.ivancic.bace@biol.pmf.hr

Insertion of new spacers into the CRISPR locus of many bacteria or archaea enables their protection from invading DNA elements. This process is called naïve adaptation and is catalysed by the Cas1-Cas2 complex only. Fragmentation of invading DNA prior to DNA insertion by Cas1-Cas2 is accomplished by RecBCD helicase activity supported by cellular single-stranded exonucleases. Consequently, naïve adaptation is strongly reduced in *recB*, *recC* or *recD* mutants. The assay for naïve adaptation requires ectopic expression of Cas1-Cas2 from the plasmid in cells lacking most of cas genes and monitoring of spacer acquisition by PCR in lysed bulk bacterial cultures after overnight growth. Spacers from certain regions of plasmids or chromosomes, such as the origin and terminus of replication or the CRISPR locus, were acquired more frequently. These regions are expected to contain more broken replication forks. The RecBCD enzyme is mainly involved in DNA repair of broken or damaged replication forks by homologous recombination, and in its absence, cell viability is greatly reduced. Therefore, in this work we wanted to test whether reduced viability affects the efficiency of adaptation in live recA recD and recB1080 cells compared to wt cells. To test this, for each strain we analysed the efficiency of adaptation, the origin of new spacers (chromosome or plasmid), the canonical or non-canonical PAM (protospacer adjacent motif), and the ability of the acquired spacers to induce autoimmunity in 10 cells that survived insertion of the new spacer. Our genetic results show that the efficiency of adaptation is not affected by the absence of recombination (reduced viability), but the number of acquired spacers with the canonical PAM or the origin of the new spacers is somewhat different than reported in the literature. Chromosomal spacers with the canonical PAM induced autoimmunity as expected.

P20

Abundance and Virulence of *Klebsiella pneumoniae* in Samples of Non-Human Origin

Ivana Kolackova, Renata Karpiskova, Kristyna Brodikova

Faculty of Medicine, Department of Public Health, Masaryk University, Brno, Czech Republic
ivana.kolackova@med.muni.cz

Klebsiella pneumoniae (KPN) has been known as a cause of disease and belongs to the most common nosocomial pathogens. On the contrary, hypervirulent KPN has emerged as a community-acquired pathogen in last few decades. It was recognized in Asia but it is spreading globally. Reservoir for human infection is usually patient's own gut but the source for initial gastrointestinal colonization remains unknown.

Limited data are available about the prevalence of KPN in animals or food therefore the aim of our study was to isolate klebsiella in food from retail, collect isolates from animals, screen those isolates for specific virulence genes and determine their phenotypic resistance to antibiotics.

Detection of KPN in food samples was performed using enrichment in buffered peptone water (Oxoid, UK) and isolation on HiCrome Klebsiella Selective Agar (HiMedia, India). Isolates from animals were collected in collaboration with clinical veterinary laboratory. Identification of KPN was confirmed using species-specific PCR (Chander et al., 2011). The presence of *iucA, ybtS, clbB* genes was screened and the virulence score 0 (low) – 5 (high) was determined based on Lam et al. (2021). Genes for capsular serotype K1, K2 were detected by PCR (Lin et al., 2014). In all isolates the string test was performed (Fang et al., 2004). Phenotypic resistance to 12 antimicrobial agents was tested and interpreted according to CLSI (2019). In selected isolates multilocus sequence typing (MLST) was performed (Brisse et al.,2009).

KPN was confirmed in 35 (50%) out of 70 samples of plant origin. The virulence score 1 was detected in seven isolates (20%), string test was positive in four isolates (11%) and only one isolate was resistant to ciprofloxacin.

Out of 61 isolates from animals (30 pigs, 31 pets), virulence score 4 was detected in 11 (18%) isolates, capsular serotype K2 was detected in six (10%) KPN and eight isolates (15%) were positive for string test. Multidrug-resistant phenotype was revealed in 10 (16%) isolates of which seven belonged to ESBL producers.

MLST typing showed high diversity in detected sequence types.

Screening for specific virulence genes and phenotype resistance to antibiotics did not confirm the spread of hypervirulent or multidrug-resistant isolates through the food of plant origin. Higher virulence score have occurred in isolates from animals, especially pigs. Even if multidrug-resistant phenotypes were found, no overlap with the virulence was detected.

P21

Phenotypic and Symbiotic Characterization of *Chickpea rhizobia* Isolated from Croatia and Herzegovina.

Ivana Rajnović[1], Sanja Kajić[1], Petra Borovec[1], Nina Toth[2], Smiljana Goreta Ban[3], Sanja Sikora[1]

[1]*University of Zagreb Faculty of Agriculture, Department of Microbiology*
[2]*University of Zagreb Faculty of Agriculture, Department of Vegetable Crops*
[3]*Institute of Agriculture and Tourism Poreč*

irajnovic@agr.hr

Sustainable agriculture without adverse impact on environment, relies on biological nitrogen fixation with emphasis on symbiotic relation between rhizobia and legumes. Screening for stress tolerant rhizobia, which are at the same time compatible with host plant and efficient in nitrogen fixation implies their identification, phenotypic characterization and effectiveness tests in field conditions. As a part of international project (PRIMA) *chickpea rhizobia* were isolated from 43 soil samples from different parts of Croatia and Herzegovina. Selected isolates were characterized by *rrs* gene sequencing and phenotypically characterized. The results showed that all isolates had good growth at elevated temperatures and at pH 4,5, 5, 6 and 9 as well as at concentrations of 1%, 2% and 3% NaCl. Two different field trials were set up in order to determine symbiotic effectiveness of selected rhizobial strains. In Poreč (Insitute of Agriculture and Tourism Poreč) three local *chickpea* cultivars were inoculated with six rhizobial strains. Significant impact on application of different rhizobial strains on nodulation was determined. The highest values both for nodule number and nodule dry weight were recorded on plants inoculated with indigenous rhizobial strain 30b and reference strain ISC11. Plants inoculated with the strain 30b had the highest N content although significant differences between different strains were not determined. The highest seed protein content was determined in plants inoculated with the same rhizobial isolate and that values were significantly higher in comparison with isolate 12b, reference strain ISC11 and control plants. The significant influence of cultivars was determined for fresh leaves biomass, seed yield, N content in plants and seeds. In the second experimental field (Faculty of Agriculture, Zagreb) one chickpea cultivar was inoculated with three rhizobial strains and intercropping with *Nigella* was encompassed. The effect of intercropping was not determined but the chickpeas response to rhizobial inoculation was significant for all measured characteristics with the exception of seed yield. Among application of indigenous and reference strain significant differences were not determined although the obtained values were higher when indigenous strain was applied. However, results obtained for total N content in plants revealed significant differences between strains whereby indigenous strain showed advantage over the reference strain.

P22

Mutations in the *Cyp51* Gene - Influence on the Resistance of *A. fumigatus* to Antifungal Compounds

Ivana Segéňová, Karin Herchlová, Petra Olejníková

Department of biochemistry and microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Bratislava, Slovakia

ivana.bacova95@gmail.com

Fungal infections affect more than a billion people every year. The origin of most serious fungal diseases are filamentous fungi of the genus *Aspergillus*, while more than 90% of aspergilloses are caused by the species *A. fumigatus*. This species was recently included in the list of priority fungal pathogens into the critical priority group by the World Health Organization. The main gateway and site of infection caused by *A. fumigatus* is the respiratory tract. Based on the immune status of the host, location and extent of colonization, these diseases can be divided into several groups characterized by different clinical manifestations. The most serious form is invasive pulmonary aspergillosis (with a mortality rate of 30-80%), in which fungi invade the lung tissue. To avoid complications, or fatal consequences, early diagnosis and the use of effective therapeutics are the key. However, the treatment of aspergillosis is significantly complicated by the resistance of pathogens to commonly used antifungal compounds. It is caused by a limited portfolio of effective antifungal compounds, as well as the use of structurally similar fungicides in agriculture.

In our work, we focused on determining the sensitivity of three clinical isolates of *A. fumigatus* (in which different resistance mechanisms were confirmed) to different antifungal compounds. Then we monitored the expression of selected genes responsible for the adaptive response by RT-qPCR. Finally, we compared the virulence of these strains on *Galleria mellonella* larvae.

This work was supported by the grant projects VEGA 1/0388/22 and APVV-19-0094.

P23

Lactogenic and Bifidogenic Effect of Innovative Mixture of Dietary Fibers for Foods for Diabetic Medical Purposes

Katarina Butorac[1], Lenkica Penava[2], Andreja Leboš Pavunc[1], Nina Čuljak[1], Martina Banić[1], Jasna Novak[1], Marijana Ceilinger[3], Jelena Miličević[3], Danijela Čukelj[2], Ivanka Jerić[4], Jagoda Šušković[1], Blaženka Kos[1]

[1]University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory for Antibiotic, Enzyme, Probiotic and Starter Cultures Technology, Zagreb, Croatia
[2]Belupo d.d., Drugs and Cosmetics Inc., Nutraceuticals, Business Development and Registration, Zagreb, Croatia
[3]Belupo d.d., Drugs and Cosmetics Inc., Nutraceuticals, Business Development and Registration, Koprivnica, Croatia
[4]Ruđer Bošković Institute, Laboratory for Biomimetic Chemistry, Zagreb, Croatia

ijeric@irb.hr

Foods for special medical purposes (FSMPs) are unique therapeutic products formulated for the specific dietary management of disease-related conditions or specific medical disorders, for which distinctive nutritional requirements, based on recognized evidence-based scientific principles, are established. The human microbiome is composed of microbes that have a tremendous potential to impact our physiology, both in health and in disease. Since high-fiber diet impacts the entire gut microbe community and improves blood-glucose regulation, they can be used as FSMPs, in which they pose a beneficial effect in maintaining the gastrointestinal health. Therefore the aim of this research was to establish the lactogenic and bifidogenic effects of three different innovative fibers (K1, D1, D2), in order to develop innovative formulations of foods for diabetic medical purposes, using a diabetic mouse model. Standard diabetic fiber mixures and food without added fibers, were used as controls. All tested innovative dietary fibers showed in vivo lactogenic and in vitro bifidogenic and lactogenic effect, where the best effects were achieved with the addition of D2 innovative fiber mixture, respectively. Additionally, after feeding the mice, all innovative dietary fibers showed a positive impact on the growth of *Lactococcus* spp., with the best results upon the addition of D2 innovative fiber mixture. Innovative fiber mixture (D1) reduced the number of *L. monocytogenes* to undetectable cell count level, significantly decreased the number of *S. aureus* and *Salmonella* spp., while innovative fiber mixture (D2) significantly decreased the number of *L. monocytogenes* and *S. aureus*, in diabetic mice faeces samples *in vivo*. The reduction of *L. monocytogenes* was also achieved with innovative (K1) and standard (K0) fiber mixtures, to undetectable cell count level. Finally, innovative fiber mixtures (D1 and D2), along with the standard fiber mixtures (K0) showed the impact on the incresement of the total bacteria number *in vivo*. In conclusion, two innovative mixtures of dietary fibers, D1 and D2, can be used as nutritional supplementation for foods for diabetic medical purposes.

P24

Plantaricin-Based Antibacterial Activity of *Lactiplantibacillus plantarum* D13 and SF15C Strains Originating from Different Food Microbiomes

Katarina Butorac, Jasna Novak, Martina Banić, Andreja Leboš Pavunc, Nina Čuljak, Jagoda Šušković, Blaženka Kos

University of Zagreb, Laboratory for Antibiotic, Enzyme, Probiotic and Starter Culture Technologies, Faculty of Food Technology and Biotechnology, Pierottijeva 6, Zagreb, Croatia

katarina.butorac@pbf.unizg.hr

In addition to being a rich source of nutrients, autochthonous fermented foods are a reservoir of beneficial lactic acid bacteria (LAB) starter cultures and potential probiotics. The bacteriocin-based antibacterial capacity of the autochthonous LAB is investigated as a strategy for biopreservation. Therefore, the aim here was to screen the antibacterial activity in a selected group of *Lactiplantibacillus plantarum* strains belonging to different food microbiomes, autochthonous cheese or sauerkraut. Since *Listeria monocytogenes* and *Staphylococcus aureus* are frequently detected as foodborne pathogens, they have been applied as test microorganisms to demonstrate antibacterial activities. All tested *L. plantarum* strains exhibited antilisterial and antistaphylococcal activities when grown on a solid medium, and when the cell-free supernatant (CFS) was analysed, where D13 and SF15C showed the greatest inhibition. Antibacterial activity in broth cultures was re-established or enhanced by coculturing with sensitive bacterial strains *S. aureus* 3048 *and L. monocytogenes* ATCC®19111™ which can be associated with the induction of bacteriocin production typically present among LAB. Additionally, the proteinase nature of CFSs has been confirmed as another feature of plantaricins. PCR using total DNA from *L. plantarum* strains resulted in the amplification of plantaricin-related genes *plnA*, *plnEF* and *plnJ* of expected amplicon size, suggesting that the genome could harbour a *pln* gene clusters. Whole genome sequencing (WGS) revealed the existence of five plantaricin specific operons regulated by a three-component regulatory system, involved in a quorum sensing (QS) mechanism, of *L. plantarum* D13 and SF15C strain, with high similarity to *L. plantarum* WCFS1. The 3D structures of PlnEF and PlnJK, were predicted using AlphaFold Protein Structure Database, while physicochemical properties and amino acid sequence of α-helixes of Pln were determined by HeliQuest analysis server. Taken together, these findings contribute to the characterisation of plantaricins of *L. plantarum* D13 and SF15C, and their implementation as either a functional starter cultures or even probiotics. Their application could improve the quality of fermented foods, which could be achieved by controlling contamination by *L. monocytogenes*, which would extend the shelf-life of the final product.

P25

Photodynamic Inactivation of *Candida albicans-Staphylococcus aureus* Biofilms in the Presence of Methylene Blue

Jarmila Czucz Varga[1], Juraj Bujdák[2,3], Helena Bujdáková[1]

[1]*Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University in Bratislava, Slovak Republic*
[2]*Department of Inorganic Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Slovak Republic*
[3]*Institute of Inorganic Chemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic*

vargova301@uniba.sk

Biofilm-associated infections represent a major threat in hospitals as they are often refractory to conventional treatment. Searching for alternative approaches to eradicate biofilm is a current challenge. Photodynamic inactivation (PDI) represents one of the alternative method that could be effective not only against single, but even against multi-species biofilms.

The effectiveness of PDI was tested against 48-h single- and dual-species biofilms (standard strains of *Candida albicans* SC5314 and *Staphylococcus aureus* CCM3953, resistant clinical isolate *C. albicans* CCY 29-3-164, methicillin-resistant *S. aureus* 6102/1/2010 -MRSA). Different concentrations of methylene blue (MB; 0.25, 0.5, and 1 mM) and duration of pre-incubation with MB (2; 4 and 16 h) were tested. Biofilms were irradiated with a red laser (190 mW/cm2, λ 660 nm, 60 s). The inhibitory effect of the PDI was determined by CFU/mL.

The effectiveness of PDI differed with respect to prokaryotic vs. eukaryotic cell structure. The most sensitive were biofilms formed by Gram-positive bacteria of *S. aureus* after 16-h pre-incubation, with a reduction of 2.08-log^10 and 1.6-log^10 (0.25 mM MB) for *S. aureus* CCM3953 and MRSA strain, respectively. The reduction in the single biofilm of *C. albicans* was only 0.4-log^10 and 0.1-log^10 for SC5314 and resistant isolate, respectively. In the case of dual biofilms, the reduction after PDI was 1.91-log^10 and 0.6-log^10 in the combination of standard strains SC5314 and CCM3953 and clinical isolates of CCY 29-3-164 and MRSA, respectively. Transformation of MB to leucomethylene blue (LMB) via reduction can affect MB photoactivity. The UV-Vis spectroscopy proved a correlation between increased LMB concentration with a longer incubation period, especially for biofilms formed by *C. albicans*. The measurement of reactive oxygen species (ROS-Glo™ H_2O_2 Assay) after 2- and 16-h- pre-incubation with MB before and after PDI proved a significant increase of ROS in biofilms of *S. aureus*, followed by mixed biofilms. The lowest ROS observed in *C. albicans* biofilms agreed with the lowest efficiency of PDI.

Result proved that biofilms of prokaryotic *S. aureus* showed significantly higher susceptibility to PDI compared to that composed of eukaryotic yeast *C. albicans*.

Acknowledgement. The research was supported by EU Grant number 952398-CEMBO, Call: H2020-WIDESPREAD-05-2020 Twinning and by the Slovak Research and Development Agency under contracts of no. APVV-19-0487 and APVV-21-0302

P26

Next-Generation of Enzyme Engineering Toward Improved Terminal Hydroxylations

Jelena Spasic[1], Berenika Stloukalová[1], Andrea Nigl[1,2], Robert Kourist[1]

[1]*Graz University of Technology, Institute of Molecular Biotechnology, Austria*
[2]*acib GmbH - Austrian Centre of Industrial Biotechnology, Austria*

jelena.spasic@tugraz.at

Terminal hydroxylation represents an attractive biocatalytic route toward terminal alcohols, aldehydes, and acids, which are of special interest as building blocks for the sustainable production of polymers. To customize biocatalysts for this purpose, several described alkane degradation pathways offer valuable starting points. Particularly interesting are alkane monooxygenases displaying a broad substrate spectrum efficiently ω-oxyfunctionalising alkanes, cycloalkanes, thioesters and fatty acid esters. In-depth knowledge of protein sequence-function relationship would enable the generation of tailored alkane monooxygenases as biocatalysts for stereo- and regioselective biotransformations and further industrial applications. However, due to the lack of crystal structures, enzyme engineering of membrane-bound alkane monooxygenases proved to be challenging. Therefore, we aim to elucidate sequence-function relationships of alkane monooxygenases using simple high-throughput screening or selection assays. The acquired data would be further used as inputs for machine learning, predicting mutational hotspots toward improved hydroxylating activities. Overall, this would result in enzyme variants showing desired activities subsequently accelerating protein engineering of alkane monooxygenases.

P27

Eradication of Staphylococcal Biofilms on New Photoactive Hybrid Film Despite Over-Regulation of the *norA* Efflux Gene

Katarína Bilská[1], Marek Pribus[1,3], Juraj Bujdák[2,3], Helena Bujdáková[1]

[1]*Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University in Bratislava*
[2]*Department of Physical and Theoretical Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava*
[3]*Institute of Inorganic Chemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic*

bilska6@uniba.sk

Staphylococcus aureus belongs to the ESKAPE group of microorganisms associated with nosocomial infections. *S. aureus* forms robust biofilms on medical devices, which can cause life-threatening infections, especially in immunocompromised patients. This study analyzed the effectiveness of a photoactive hybrid film (HF), functionalized on polyurethane (PU), composed of clay mineral saponite (Sap) modified with poly(diallyldimethylammonium) (PDDA) and with photosensitizer phloxine B (PhB).

For experiments, standard strain *S. aureus* CCM3953 and methicillin-resistant *S. aureus* (MRSA) L12 were used. The material was tested against 24-h biofilms before and after irradiation using a green laser (λ = 532 nm, 100 mW, 120 s). Using E-tests, strain L12 was confirmed to be resistant to oxacillin, ciprofloxacin, and norfloxacin (MIC > 256 μg/mL, MIC > 32 μg/mL, MIC > 256 μg/mL, respectively), while CCM3953 was sensitive to all tested drugs. The presence of the PIA adhesin encoded by the *icaA, icaB, icaC, icaD* genes, and the negative regulator *icaR* were confirmed in both strains by PCR.

The efflux activity assessed by the ethidium bromide agar screening method showed to be higher for strain L12 compared to control strain CCM3953. Regulation of the *NorA* gene in 24-h biofilm formed on PU without modification was determined by quantitative PCR (the $2^{-\Delta\Delta CT}$ method). Results showed the 7.51 times higher expression for strain L12 compared to CCM3953 set to 1. The expression of the *NorB* gene was not examined.

Preliminary testing of new HF was performed only with standard strain CCM3953 on polytetrafluoroethylene membrane modified with HF containing PhB. HF prepared in different ratios of PhB/Sap (nPhB/mSap: 0.5 mmol/g, 1.0 mmol/g, and 1.5 mmol/g) demonstrated 2.03 log^10; 3.37 log^10; and 9.02 log^10 (100%) inhibition after photodynamic inactivation, respectively. Based on these results, HF containing PhB/Sap in ratio (nPhB/mSap) 1.5 mmol/g was functionalized on

PU. Results showed 2.84 log^10 and 2.64 log^10 inhibition after PDI for CCM3953 and MRSA L12, respectively.

Results suggest a promising potential of HF with photoactive properties in eradication of staphylococcal biofilms, even in case of MRSA biofilm with the up-regulated *NorA* gene.

Acknowledgement. This work was supported by the Slovak Research and Development Agency under contracts of no. APVV-18-0075 and APVV-21-0302.

P28

Antifungal and Antibacterial Effect of Green Tea Catechins

Katarína Hollá[1], Lucia Bírošová[1], Petra Olejníková[2]

[1]*Department of Nutrition and Food Quality Assessment, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava*
[2]*Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava*

katarina.holla@stuba.sk

Catechins are natural tea flavonoids known for their antioxidant, anti-inflammatory and antimicrobial properties. There are many studies that show the potential of catechins as scavengers of reactive oxygen species and therefore they protect cells against an oxidative stress. On the other hand, catechins can act as prooxidants in higher concentrations; they can damage bacterial membranes and thus have an inhibitory activity against both gram-negative and gram-positive bacteria. Therefore they are likely to have promising applications in antimicrobial therapy in potentiating the activity of antibiotics. These applications may be interesting mainly in the fight against resistant bacterial strains, but there is still a need for more scientific investigation. In our research, we tested the antifungal effect of catechin-hydrate against model organism *Neurospora crassa* and its effectiveness in combination with azole derivates in different concentrations. As catechins could show prooxidative activity in applied higher concentrations, we also monitored an expression of genes involved in cellular response to oxidative stress in *Neurospora crassa* in the presence of catechins. The antibacterial effect of catechins was tested on different *Escherichia coli* strains, both sensitive and resistant to antibiotics. Our goal was to identify an inhibitory activity of catechins against fungal and bacterial model organisms and to find a potential synergistic effect between catechin and certain antimicrobial drugs that can be beneficial in the treatment of infections caused mainly by resistant bacterial strains.

This work was supported by the Slovak Research and Development Agency under the Contract No. APVV-19-0094 and project No. VEGA 1/0388/22 and by the Slovak Research.

P29

Intracellular Protein Targets of *Neosartorya* (*Aspergillus*) *fischeri* Antifungal Proteins

Kinga Dán[1], Zoltán Kele[2], Liliána Tóth[1], Fanni Marcsisák[1], Gábor K. Tóth[2,3], Gábor Rákhely[1,4], László Galgóczy[1,5]

[1]*University of Szeged, Faculty of Science and Informatics, Department of Biotechnology, Szeged, Hungary*
[2]*University of Szeged, Albert Szent-Györgyi Medical School, Department of Medical, Chemistry, Szeged, Hungary*
[3]*University of Szeged, MTA-SZTE Biomimetic Systems Research Group, Szeged, Hungary*
[4]*Eötvös Loránd Research Network, Biological Research Centre, Institute of Biophysics, Szeged, Hungary*
[5]*Eötvös Loránd Research Network, Biological Research Centre, Institute of Biochemistry, Szeged, Hungary*

dan.kinga@bio.u-szeged.hu

Nowadays the incidence of fungal infections caused by antifungal drug-resistant strains is continuously increasing. Therapeutic application of antifungal proteins from the ascomycete, *Neosartorya* (*Aspergillus*) *fischeri* (NFAP and NFAP2) may overcome this problem. These proteins have favorable pharmacological features such as the small molecular weight, highly stable structure, and high solubility in different solvents. Our previous studies indicated that NFAP is effectively inhibit the growth of *Aspergillus fumigatus* isolates *in vitro*, meanwhile the NFAP2 the growth of antifungal drug-resistant Candida albicans isolates both *in vitro* and *in vivo*. Understanding the mode of action of NFAP and NFAP2 by investigating their molecular targets in fungal cells is required for a reliable therapeutic application, therefore, in the present study we investigated the intracellular protein targets of NFAP and NFAP2 in *A. fumigatus* and *C. albicans*, respectively. We also investigated their protein targets in *N. fischeri* to reveal the biological function in the native producer. We applied different *in vitro* mass spectrometry-coupled protein-protein interaction detection methods to identify the intracellular protein targets of NFAP and NFAP2. For the *in vitro* farwestern blotting assay, and the label transfer protein interaction analysis we used the intracellular total protein extracts of *A. fumigatus* CBS 101355, *C. albicans* SC5314, and *N. fischeri* NRRL 181. The results with NFAP suggest that this protein interacts with cytosolic (e.g. mannitol-1-phosphate dehydrogenase, transaldolase, Hsp70) and mitochondrial (e.g. malate dehydrogenase, glutamate dehydrogenase) enzymes and proteins, which may influence the basic metabolism both of *A. fumigatus* and *N. fischeri*. Our results indicated that NFAP2 can interact with triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, and enolase 1 of *C. albicans*. These

proteins are important enzymes of glycolysis. We observed that NFAP2 is able to interact with superoxide dismutase and a conserved lysine-rich protein of *N. fischeri*. The conserved lysine-rich protein may be involved in targeting the protein to the cellular location. Further *in vivo* investigations are in progress to prove the reliability of these in vitro protein hits.

L.G. was financed by the Hungarian National Research, Development and Innovation Office -NKFIH, FK 134343 project.

P30

Microplastics and Their Interactions with Microorganisms and Mobile Genetic Elements

Klára Cverenkárová[1], Petra Olejníková[2], Tomáš Mackuľak[3], Lucia Bírošová[1]

[1]*Institute of Food Science and Nutrition, Faculty of Chemical and Food Technology STU in Bratislava, Slovakia*
[2]*Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology STU in Bratislava, Slovakia*
[3]*Institute of Chemical and Environmental Engineering, Faculty of Chemical and Food Technology STU in Bratislava, Slovakia*

klara.cverenkarova@stuba.sk

Microplastics represent a new source of pollution in the environment. Their occurrence has been recorded in water, soil, seas, and oceans. When researching microplastics, it was soon discovered that they can interact with environmental components, such as pollutants or microorganisms. Antibiotic resistant bacteria can also be a part of the microplastics biofilm. The effect of microplastics on resistant bacteria is still relatively unknown. In this work, we monitored the influence of selected microplastics on the development of mutations leading to ciprofloxacin resistance in model bacteria *Salmonella typhimurium* using a modified Ames test. The absorption capacity of microplastics for the plasmid carrying antibiotic resistance genes (pRS426) was assessed with a simple experiment.

Acrylonitrile butadiene styrene (ABS), polylactic acid (PLA) microplastics, both 0.09 and 0.125 mm in size and PET microplastics (0.125 mm) were used. Microplastics were added to the bacterial culture in different dosages from 5 to 50 mg. From the results, it can be concluded that most of the microplastics used and their concentrations did not have a significant impact on the development of mutations leading to ciprofloxacin resistance. The frequency of resistant mutants was at the same level or lower as the control with no added microplastics. We observed a slight increase in the frequency of resistant mutants with addition of 10 mg of ABS (0.09 mm) and 5, 10 and 50 mg of PLA (0.09 mm) and 5 mg of PET microplastics but the ratio of frequency of resistant mutants to the control did not exceed the value of 2.90.

The absorption experiment with plasmid DNA and all of the microplastics did not show any significant results. The DNA concentration in solution with microplastics decreased only in case of PLA microplastics in the size of 0.09 mm and PET microplastics.

The authors would like to thank for financial contribution from the STU Grant scheme for Support of Young Researchers no. 1894 and project VEGA 1/0464/21.

P31

Application of *Candida parapsilosis* in Biodegradation of UV/S2O82--pretreated MP-PS

Kristina Bule Možar, Martina Miloloža, Viktorija Martinjak, Matija Cvetnić, Šime Ukić, Tomislav Bolanča, Dajana Kučić Grgić

University of Zagreb Faculty of Chemical Engineering and Technology, Zagreb, Croatia

kbule@fkit.hr

Microplastic (MP), particles ranging in size from 1 μm to 5 mm, are of concern due to their ubiquity in the environment. MP have harmful effects on living organisms and the environment in general. Various methods are being explored to remove these particles from the environment.

In this study, polystyrene microplastic (MP-PS) were pretreated with UV/S2O82- and then used in a biodegradation process with the yeast *Candida parapsilosis*. The optimal UV/S2O82- conditions were determined using a full factorial experimental design with three parameters (pH of the medium, S2O82- oxidant concentration, and exposure time) in three levels. Before and after each process, the MP-PS particles were characterized by FTIR spectroscopy. After processing the data, the optimal conditions for the degradation of MP-PS by the UV/S2O82- process are the exposure time of 83.06 min at a concentration of S2O82- of 9.72 mM and a pH of the medium of 7.45. After the pretreatment of MP-PS under the above conditions, the processed MP was used for the biodegradation process, and a biodegradation experiment was also conducted with MP-PS, which had not been pretreated. The biodegradation experiments lasted for 30 days. During the experiment, the number of live yeast cells (CFU) and the concentration of total, organic and inorganic carbon (TC, TOC and TIC) were monitored. Considering the change in CFU over 30 days, it was concluded that biodegradability was improved by pretreatment of PS.

P32

Heterologous Expression and *in vitro* Antifungal Effect of a Novel *Solanum lycopersicum* L. Antifungal Defensin

Rebeka Papp[1], Györgyi Váradi[2], Zoltán Kele[2], Gábor Rákhely[1,3], Péter Poór[4], Gábor K. Tóth[2,5], László Galgóczy[1,6], Liliána Tóth[1]

[1]*Department of Biotechnology, Faculty of Science and Informatics, University of Szeged, Hungary*
[2]*Department of Medical Chemistry, Albert Szent-Györgyi Medical School, University of Szeged, Hungary*
[3]*Institute of Biophysics, Biological Research Centre, Eötvös Loránd Research Network, Hungary*
[4]*Department of Plant Biology, Faculty of Science and Informatics, University of Szeged, Hungary*
[5]*MTA-SZTE Biomimetic Systems Research Group, University of Szeged, Szeged, Hungary*
[6]*Institute of Biochemistry, Biological Research Centre, Eötvös Loránd Research Network, Hungary*

toth.liliana88@gmail.com

Recently, the control of the enormous crop losses caused by filamentous fungal infections is a serious challenge for agriculture. One of the reasons is the increasing number of fungicide-resistant strains. This problem is aggravated by the European Commission, which initiated the reduced chemical pesticide application in agriculture, and their withdraw from the market. Therefore, there is an urgent need for new fungicide molecules with a different antifungal mode of action that the chemical ones have. Antifungal plant defensins are considered as new biofungicide molecules as they effectively inhibit the growth of several plant pathogenic fungi; however, their agricultural application is limited by the fact that plants produce them in very low amount. To overcome this problem, in the present study, we developed a *Pichia pastoris*-based heterologous expression system for the bulk production of a novel *Solanum lycopersicum* L. defensin (K4CBP6), and investigated its antifungal effect.

The generated K4CBP6-producer *P. pastoris* strain secreted the defensin into the supernatant which allowed an easy, single-step cation exchange chromatographic purification. The average yield of the recombinant K4CBP6 was 8.4 ± 0.5 mg l−1 (n = 3). Mass spectrometry and high-performance liquid chromatography analyses proved that the heterologous K4CBP6 is correctly processed.

An *in vitro* microdilution susceptibility test indicated that K4CBP6 effectively inhibit the growth of *Botrytis cinerea* SZMC 21472, *Cladosporium herbarum* FSU 1148 and several *Fusarium* strains with minimum inhibitory concentration of 25-12.5 μg/ml. In contrast, K4CBP6 was ineffective against the tested *Aspergillus* strains.

In conclusion, the developed *P. pastoris* expression system can be applicable for the bulk production of the promising biofungicide molecule, K4CBP6 after the optimization of the fermentation conditions.

Present work of L.T. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH, PD 134284 project.

P33

Comparative Genomic Analysis Reveals Low Association Between Genetic Content and Ropy Phenotype of *Rahnella inusitata* and *Klebsiella pneumoniae* Subsp. *pneumoniae*

Lucija Podrzaj[1], Renato H. Orsi[2], Nicole H. Martin[2], Martin Wiedmann[2], Aljoša Trmčić[2]

[1]*Institute of Food Science, Department of Food Science and Technology, University of Natural Resources and Life Sciences, Vienna, Austria*
[2]*Department of Food Science, Cornell University, Ithaca, New York, United States of America*

lucija.podrzaj@boku.ac.at

Ropy milk is an important quality defect and a spoilage sign in fluid milk. This spoilage is caused by Gram-negative bacteria which can be introduced in milk as post-pasteurization contaminants (PPC) and produce exopolysaccharides (eps). However, limited information on bacterial species and strains, their phenotypic properties and ultimately specific genes that may be attributed to spoilage incidents are available. This study aimed to investigate the genomes of five bacterial strains including *Rahnella inusitata* (2) and *Klebsiella pneumoniae* subsp. *pneumoniae* (3) obtained from samples of pasteurized milk (n=3), raw milk (n=1) and yogurt (n=1) to determine the genetic basis for ropy phenotypes. Growth analysis in UHT milk revealed increase of bacterial counts of all strains by at least 4-log over 48 h at 21 °C, but only two *K. pneumoniae* subsp. *pneumoniae* strains and one *R. inusitata* strain were capable to cause spoilage of milk revealed by increase in viscosity and formation of long filaments. At 6 °C, the growth was observed for *R. inusitata* only, with one *R. inusitata* strain being able of causing ropy spoilage of milk. Considering the viscosity of milk, the three strains capable of causing ropy defect in milk at 21 °C revealed various degrees of viscosity changes during growth in milk. Comparative genomics identified eps gene clusters in genomes of all strains; however, clusters of eps biosynthesis genes were organized in different ways. Furthermore, no clear-cut relationship between the ropy phenotype in milk and gene contents were found when strains of the same species were compared. Overall, although the potential of *R. inusitata* and *K. pneumoniae* subsp. *pneumoniae* to cause a ropy defect in milk have been identified as strain-dependent, relationship between genetic background and observed phenotypes is not well understood and merits further investigation.

P34

Antioxidant Properties of *Lactobacillus casei* N87 for Fermented Sausages Production

Lucilla Iacumin, Michela Pellegrini, Giuseppe Comi

Department of Agricultural, Food, Environmental and Animal Science, University of Udine, Italy
lucilla.iacumin@uniud.it

Lcb. casei N87, cultivated under anaerobic (A) and respiratory (R) conditions, were used as starter cultures for the production of fermented sausages. Sausages produced without the starter culture inoculation and with the addition of 150 mg/kg of nitrate were also included as controls. The effect on physico-chemical parameters (pH, Aw, weight loss, and color), microbial population, volatilome, proteolysis as well as the survival of the strain was evaluated during 90 days of ripening. Q-PCR and DGGE-PCR analyses demonstrated the ability of the strain used in this study to adapt to the environment and carry out the sausage's fermentation process. The inoculation of the strain didn't show any effect on the Aw values, which decreased similarly in the different samples. Conversely, pH was lower in A samples (5.2) and the weight loss in R samples (2.5% less than the others). The color parameters of the samples inoculated with the starter cultures were comparable to those of the control added with nitrate. The concentration of aldehydes that usually are identified as marker of oxidation processes was similar in the samples inoculated with the starter cultures adapted under respiratory conditions and in the control. On the contrary, a higher level was detected in the samples inoculated with the starter cultivated under anaerobic conditions. The proteolysis that occurred during the ripening indicates the differentiation of the A samples from the others. Nonetheless, the volatile profiles of the inoculated fermented sausages were similar. This study demonstrated the possibility to eliminate the use of nitrate in fermented sausage production using *Lcb. casei* N87 starter culture adapted under respiratory conditions without affecting the color parameters and the amount of aldehydes related to oxidative processes.

P35

Can *Lacticaseibacillus paracasei* Strains Reduce the Duration of Respiratory Tract Infections?

Maja Strauss[1], Dušanka Mičetić-Turk[1,2], Mateja Lorber[1], Sabina Fijan[1]

[1]*Faculty of Health Sciences, Institute for Health and Nutrition, University of Maribor, Maribor, Slovenia.*
[2]*Faculty of Medicine, University of Maribor, Maribor, Slovenia.*

maja.strass@um.si

Introduction: Various agents can cause respiratory infections, mainly bacteria and viruses. Respiratory infections include infections of the upper and lower respiratory tract. Colds and flu are the most common, whilst pneumonia causes the greatest mortality, especially in the older population. Other common respiratory diseases are nose, throat, trachea, and lung infections. Respiratory infections are more common in the autumn and winter seasons. In adults they are associated with absence from work and high treatment costs. The aim of this narrative review was to determine if probiotics *Lacticaseibacillus paracasei* strains can reduce the duration of respiratory tract infection.
Methods: We performed a review to investigate the influence of probiotics on the duration of acute upper respiratory-tract infections in adults, by regulating the immune system. Five randomized, placebo-controlled clinical trials met the inclusion criteria, considering the threshold of being 18 years and over. We searched PubMed using the keywords: 'PROBIOTICS' and 'RESPIRATORY TRACT INFECTIONS'. Studies investigating children and postbiotics were excluded. Only studies available in full text and in English language that reported duration of respiratory tract infections were included.
Results: The following single strain probiotics were used: *Lacticaseibacillus paracasei* subsp. *paracasei* CNCM I-1518, *Lacticaseibacillus paracasei* subsp. *paracasei* 431 and *Lacticaseibacillus paracasei* Shirota. Included research show significantly lower duration of all pathologies and duration of respiratory tract infection. The review of the literature shows that these probiotics can improve gut-lung axis.
Conclusion: Current evidence showed that all probiotic strains of *Lacticaseibacillus paracasei* were better than placebo in lowering the duration of acute upper respiratory tract infections in adults. Thus, proving the importance of species-specific effectiveness of probiotics. More high quality large-scale properly controlled clinical studies are warranted.

P36

The Impact of the Co-Treatment of Municipal Wastewater and Landfill Leachate on the Changes in Microbial Biodiversity and Spread of Multidrug Resistance

Małgorzata Czatzkowska, Monika Harnisz

Department of Water Protection Engineering and Environmental Microbiology, University of Warmia and Mazury in Olsztyn, Poland

malgorzata.czatzkowska@uwm.edu.pl

Waste disposal in landfills entails the generation of landfill leachate (LL). The process of water percolating through layers of deposited waste leads to the leaching of microorganisms, including pathogens, as well as high content of organic matter, heavy metal ions, and salts. The popular solution to the problem of LL management poses the combined treatment of LL with wastewater in municipal wastewater treatment plants (WWTPs). Due to its toxicity, the systematic presence of LL in the WWTPs treatment system may influence the wastewater microbial composition. Moreover, both landfills and WWTPs create environments, particularly predisposing to processes of the exchange of genetic structures, including antibiotic resistance genes (ARGs), among microorganisms. It is known that *mexF* gene, which encodes multidrug efflux pumps, is characteristic for landfills and LL. Importantly, the spread of multidrug resistance determinants is crucial for environmental pollution and public health. Considering the above, our study aimed to determine how the co-treatment of toxic LL and municipal wastewater influences the changes in wastewater biodiversity and the spread of multidrug resistance.

Therefore, we present the results of studies conducted simultaneously in two similar WWTPs, one of which receives LL and co-treats it with municipal wastewater, and the other one does not collect LL. The material for the analyses consisted of (1) LL delivered to a WWTP from a landfill, (2) samples of influent, and (3) effluent from each of the two WWTPs. Using molecular methods (metagenomic sequencing and ddPCR), the characteristics of the microbial structure and the concentration of the *mexF* gene in the samples were determined. The study showed, that the predominant bacterial phyla in LL were *Firmicutes* (42.4%), *Bacteroidota* (26.5%), and *Proteobacteria* (11.8%). The analysis of the microbial co-occurrence network in the wastewater showed that exposure to LL induced changes in interactions between microbial taxa in wastewater and posed a stress factor to bacteria. The concentration of the *mexF* gene was determined at 101 copies/mL in LL. That value was two and four orders of magnitude higher than in untreated and treated wastewater samples, respectively. The study provides that LL poses a reservoir of multidrug determinants. Moreover, the *mexF* gene could be identified as a potential marker of LL pollution in the environment.

P37

Effect of *Trichoderma* on the Degradation of Chitosan Films Modified by Phenolic Acids

Maria Swiontek Brzezinska[1], Grażyna B. Dąbrowska[2], Beata Kaczmarek-Szczepańska[3], Katarzyna Dembińska[1], Marcela Pejchalová[4], Sweta Binod Kumar[1], Agnieszka Richert[2], Agnieszka Kalwasińska[1]

[1]*Department of Environmental Microbiology and Biotechnology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Torun, Poland*
[2]*Department of Genetics, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Torun, Poland*
[3]*Department of Biomaterials and Cosmetics Chemistry, Faculty of Chemistry, Nicolaus Copernicus University in Torun, Torun, Poland*
[4]*Department of Biological and Biochemical Sciences, University of Pardubice, Pardubice, Czech Republic*

swiontek@umk.pl

The aim of the study was to determine the potential use of fungi of the genus *Trichoderma* for the degradation of phenolic acid-modified chitosan in compost. At the same time, the enzymatic activity in the compost was checked after application of a preparation containing the fungi *Trichoderma*. The *Trichoderma* strains were characterized by high lipase and aminopeptidase activity, chitinase, and 1,3 β-glucanases. *T. atroviride* TN1 and *T. citrinoviride* TN3 metabolized the modified chitosan films best. Biodegradation of modified chitosan films by native microorganisms in the compost was significantly less effective than after the application of a formulation composed of *Trichoderma* TN1 and TN3. Bioaugmentation with a *Trichoderma* preparation had a significant effect on the activity of all enzymes in the compost. The use of *Trichoderma* consortium significantly increased the chitinases activity.

P38

The Influence of Tannic Acid on the Antimicrobial and Biological Properties of Collagen/Beta Glucan Hydrogels

Marta Michalska-Sionkowska[1], Oliwia Warżyńska[1], Krzysztof Łukowicz[2], Anna Maria Osyczka[2], Maciej Walczak[1]

[1]Faculty of Biological and Veterinary Sciences, Department of Environmental Microbiology and Biotechnology, Nicolaus Copernicus University in Toruń, Toruń, Poland
[2]Institute of Zoology and Biomedical Research, Department of Biology and Cell Imaging, Faculty of Biology, Jagiellonian University, Kraków, Poland

mms@umk.pl

Natural polymers that are produced by living organisms serve as biodegradable matrices that contain repeating units linked together by covalent bonds [1]. As they have many advantages like biocompatibility, biodegradability, and non-toxicity, they are used as biomaterial components [2]. In this study, hydrogels of collagen β-glucan modified with tannic acid were obtained in neutralization process as a potential wound dressing material. An open wound is a niche that is favourable for microbial colonization. Therefore, the current trend is to develop materials with antimicrobial properties to prevent bacterial infection. It is crucial that the material is not toxic to somatic cells while being antimicrobial. In this study tannic acid (TA) was used as a crosslinking agent (which provided better mechanical properties) and was released from matrices as an antimicrobial agent. Materials affected on dehydrogenase activity and ATP level of pathogens and the effect was dose depended. The biocompatibility study was performed using human keratinocytes' (HaCaT cell line). The extracts obtained from the studied hydrogels increased the HaCaT cells' viability by 20–60% compared to TCP, depending on the tannic acid content. The highest cell viability could be observed for hydrogels with 10% TA, and it was significantly higher than for the respective hydrogels with 2 and 5% of TA [3]. Therefore, using tannic acid as a component of materials may prove effective not only as a crosslinking agent but also as an antimicrobial agent that does not show cytotoxicity to human cells.

Acknowledgments. Authors are grateful for financial support from the National Science Centre, Poland, Grant nr 2018/31/N/ST8/01509.

[1] Bhargava Reddy, M.S.; Ponnamma, D.; Choudhary, R.; Sadasivuni, K.K. A Comparative of Natural and Synthetic Biopolymer Composite Scaffold. Polymer 2021, 13, 1105.
[2] Sionkowska, A.; Adamiak, K.; Musiał, K.; Gadomska, M. Collagen Based Materials in Cosmetic Applications: A Review. Materials 2020, 13, 4217.
[3] Michalska-Sionkowska, M.; Warżyńska, O.; Kaczmarek-Szczepańska, B.; Łukowicz, K.; Osyczka, A.M.; Walczak, M. Characterization of Collagen/Beta Glucan Hydrogels Crosslinked with Tannic Acid. Polymers 2021, 13, 3412.

P39

Implementation of Microbial Source Tracking Using Multiplex Digital PCR in Slovenia

Matjaž Retelj, Petra Vovko, Tanja Žlender, Maja Rupnik, Katja Zelenik

National Laboratory for Health, Environment and Food, Slovenia

matjaz.retelj@nlzoh.si

Faecal pollution of water presents a public health risk. Faecal pollution is determined by detecting faecal indicator bacteria. However, these bacteria inhabit the digestive tracts of very different animal species, and their presence in water provides no information about the source of pollution. Identifying the pollution sources is critical for risk assessment and the implementation of remedial action. For that reason, microbial source tracking (MST) methods have been developed. The most reliable of them are molecular methods that detect markers of microbes that inhabit the intestines of specific animal host species. MST markers must be selected and validated for each geographical area of use due to the diversity of animal species and breeds, as well as the locally available feed for them. In our research, we developed the first molecular MST method in Slovenia. The new method is based on a multiplexed digital polymerase chain reaction. We validated it on 84 faecal samples from various animals and determined that it is specific and sensitive for different animals. The method corresponded very accurately with the nominal values of a standardized reference material from the US NIST. Parallel testing of water from areas where the source of faecal pollution can be predicted relatively well, with standard bacteriological methods for faecal indicator bacteria and with the new method demonstrated that the detected molecular markers are consistent with the results of the standard methods and the expected and most probable sources of faecal pollution. We have shown the method reliably determines faecal pollution from humans, ruminants (cattle, sheep, deer, goats), cattle, pigs, or birds. Its additional advantage is that it also quantifies the contributions of these animal species to the pollution. The prevalence of faecal water pollution in Slovenia highlights the necessity to broaden the range of analytical techniques available to aid in the remediation of polluted water sources and water bodies.

P40

Antioxidative and Antimicrobial Properties of the Medicinal Plant *Amorphophallus shyamsalilianum*

Miklós Takó[1], Tamás Kovács[1], Muhammad Iqbal Perdana[1], Bernard Gitura Kimani[1], Mónika Varga[1], Erika Beáta Kerekes[1], Kumeera Bethala[2], Swapnil Bhimrao Matikhaye[2], Subhash R. Somkuwar[3], Jagannath V. Gadpayale[4], Pramod W. Ramteke[2], Csaba Vágvölgyi[1]

[1]*Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary*
[2]*Department of Biotechnology, Dr. Ambedkar College, Deekshabhoomi, Nagpur, India*
[3]*Department of Botany, Dr. Ambedkar College, Deekshabhoomi, Nagpur, India*
[4]*Department of Botany, S. N. Mor College of Arts, Commerce & Smt. G. D. Saraf Science College, Tumsar (M.S.), India*

tako78@bio.u-szeged.hu

Many plants are excellent reservoir of bioactive molecules having beneficial effects on the human body. Phenolic compounds of plants, for instance, can exert various health protective effects such as anti-inflammatory, anticancer, and antimicrobial activities. Several plants or plant residues are used traditionally in the folk medicine, and many of them have been characterized from their bioactive properties. However, there are several plants, including the *Amorphophallus shyamsalilianum*, that have not been characterized from this aspect. The main goal of this project is to investigate and characterize the bioactive properties, e.g., phenolic content, antioxidant and antimicrobial activities, of the ethnomedicinal and endemic plant *A. shyamsalilianum* collected from India.

Various residue parts, i.e., leaf, petiole and corm of the plant were chopped, and after a lyophilization the dried materials were grinded to about 1-3 mm diameter size. The extraction for soluble phenolics was conducted by a methanol-based solvent treatment combined with ultrasound extraction. For bound phenolics, i.e., the compounds that are in glycoside bonds, an alkaline extraction approach was also carried out. The extracts then were subjected to phenolic content and antioxidant activity determination using spectrophotometric methodologies. Total phenolic content analyses detected high soluble phenolic yield in the leaf residue, while the leaf and petiole materials proved to be excellent reservoirs for bound phenolics. In all residues, overall yield of bound phenolics was higher to those detected for soluble molecules. In addition, the DPPH, FRAP, CUPRAC and ORAC approaches used to determine antioxidant activity showed improved antioxidative properties in extracts containing phenolics released from their bound form. Effect of extracts against the growth of *Escherichia*, *Bacillus*, *Salmonella*, *Staphylococcus*, *Pseudomonas*, *Saccharomyces* and *Debaryomyces* food-contaminating microorganisms was also evaluated in microdilution experiments. Extracts of leaf and petiole residues generally showed a good inhibitory potential towards the bacteria studied.

This research was supported by the grants NKFI FK 134886 and TKP-2021-EGA-28.

P41

Diversity of *Pseudomonas* spp. Isolated from Carst Cave

Mirna Mrkonjic Fuka[1], Irina Tanuwidjaja[1], Lucija Podrzaj[1], Johanna Brutscher[2], Konrad Domig[2]

[1]*Faculty of Agriculture University of Zagreb, Zagreb, Croatia*
[2]*University of Natural Resources and Life Sciences, Vienna, Austria*

mfuka@agr.hr

Dinaric karst region extending from Soča river in Italy to Prokletije Mt. between Montenegro and Albania is considered to be the richest in cave biodiversity in the world. However, there is limited number of research on cave microbiota from Croatian karst caves. Up to now, microbial investigations of cave environments mostly focused on *Actinobacteria*, while there is a lack of knowledge on *Pseudomonas* species isolated from caves. Therefore, the aim of this study was to isolate and identify *Pseudomonas* spp. from cave sediments and water and to determine their intraspecies diversity as well as pattern of antibiotic resistance and antimicrobial activity. Cultivable microbiota was isolated from sediments and water (n = 19) that were collected from karst cave located near Šibenik at three different timepoints. The individual, randomly selected colonies were purified and identified by MALDI-TOF (n = 322). Bacteria identified as *Pseudomonas* spp. (n = 63), was genotyped and selected by rep-PCR and their resistance pattern against six classes of antibiotics as well as antimicrobial activity against potentially pathogenic microorganisms in agriculture (n = 9) was determined. High inter- and intraspecies variability was found among isolated pseudomonads. About 11% of *Pseudomonas* spp. were resistant to two classes of antibiotics and 1,6% were multidrug-resistant (MDR). Antimicrobial activity was noticed against all tested pathogens, however, the effect was species and strain dependent. Most of the *Pseudomonas* strains completely inhibited the growth of *Erwinia amylovora* and *Listeria innocua*.

P42

Mycoflora of Forest Tree Species in Continental and Mediterranean Zones

Nevenka Ćelepirović[1], Sanja Novak Agbaba[1], Monika Karija Vlahović[2]

[1]*Croatian Forest Research Institute*
[2]*University of Zagreb, School of Medicine, Department of forensic medicine and criminology, DNA Laboratory*

nevenkc@sumins.hr

Forest trees display a variety of symptoms of diseases caused by fungi. The goal of the research was to determine the biodiversity of diseases caused by fungi on forest trees. The samples was collected in the forest of the management units East Psunj and Papuk Nature Park and Učka Nature Park in Croatia. The fungi were identified using a combination of disease symptoms, fruiting body morphology, fungal culture, and DNA barcoding. On the samples of plant material, the symptoms of the disease were analyzed using diagnostic characteristics, and the identification of the causative agent of the disease was carried out according to the symptomatology and descriptions in the literature for certain types of fungi. Infected parts of the plant material were placed in moist conditions on filter paper to develop fruiting bodies for analysis, and on a culture media (PDA, MEA, etc.) to obtain a fungal culture for macroscopic and microscopic examination for determination of the morphological characteristics of the fungi. DNA samples for DNA barcoding were isolated from infected leaves, branches, trunks, and bark, and fungal cultures. Molecular identification of the fungi was provided using primary (the nuclear ribosomal internal transcribed spacer (ITS)) and secondary (the translation elongation factor 1α, TEF1α) fungal DNA barcode regions. After determining the reference sample in the GenBank (NCBI), all sequences were registered to the GenBank (NCBI) under a unique accession number. The following saprotrophic, endophytic, and parasitic fungal species were detected: *Alternaria alternate, Amphilogia gyrosa, Apiognomonia errabunda, Asteroma coryli, Aureobasidium pullulans, Biscogniauxia mediterranea, Capronia pilosella, Cladosporium* sp., *Colletotrichum lineola, Coprinellus micaceus, Cristulariella depraedans, Cristulariella depraedans, Cryphonectria parasitica, Cyclaneusma* spp., *Cytospora* sp., *Diatrypella pulvinata, Didymella* sp., *Digitodochium amoenum, Diplodia sapinea, Dothiorella symphoricarposicola, Epicoccum nigrum, Epithamnolia xanthoriae, Exidia glandulosa, Fusarium* spp., *Gloeosporium coryli, Gnomoniella carpinea, Gnomoniella fraxini, Melanops fagicola, Mycosphaerella maculiformis, Mycosphaerella pini, Mycosphaerella* spp., *Nectria* spp., *Penicillium glabrum, Phyllactinia* spp., *Rhytisma* spp., *Rosellinia corticium, Sphaerognomonia carpinea, Sphaeropsis sapinea, Sydowia polyspora, Trichoderma deliquescens, Trichothecium roseum* and *Venturia tremulae*.

P43

Aeromonas spp. as Reservoirs of Clinically Relevant Antibiotic Resistance in the Aquatic Environment

Nikolina Udiković Kolić[1], Sara Drk[1], Ana Puljko[1], Mia Dželalija[2]

[1]*Ruđer Bošković Institute, Zagreb, Croatia*
[2]*Faculty of Science, University of Split, Split, Croatia*

nudikov@irb.hr

Antibiotic resistance (AR) remains one of the greatest threats to global health, and Aeromonas species have the potential to spread AR in the aquatic environment. The spread of resistance to antibiotics important to human health, such as third generation cephalosporins (3GCs) and carbapenems, is of great concern. We isolated and identified 15 cefotaxime (3GC)- and 51 carbapenem-resistant *Aeromonas* spp. from untreated hospital and treated municipal wastewater in January 2020. The most common species were Aeromonas caviae (58%), *A. hydrophila* (17%), *A. media* (11 %), and *A. veronii* (11 %). Almost all isolates exhibited a multidrug-resistant phenotype and harboured a diverse plasmidome, with the plasmid replicons ColE, IncU, and IncR being the most frequently detected. The most prevalent carbapenemase gene was the plasmid-associated *blaKPC-2*, and for the first time, the *blaVIM-2*, *blaOXA-48*, and *blaIMP-13* genes were identified in *Aeromonas* spp. Among the 3GC-resistant isolates, the *blaGES-5* and *blaMOX* genes were the most prevalent. Of the 10 isolates examined, three were capable of transferring carbapenem resistance to susceptible recipient *E. coli*. Our results suggest that potentially pathogenic *Aeromonas* spp. can act as reservoirs for clinically significant AR in the environment and thus pose a threat to both the environment and public health.

P44

In Vitro Testing of SARS-CoV-2 E Protein Specific Inhibitors Selected from *in Silico* Studies

Nina Kobe[1], Črtomir Podlipnik[2], Lennart Dreisewerd[2], Marko Jukić[3], Polona Kogovšek[1]

[1]*National Institute of Biology, Slovenia*
[2]*Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia*
[3]*Faculty of Chemistry and Chemical Technology, University of Maribor, Slovenia*

nina.kobe@nib.si

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is constantly evolving and mutating over time into newer variants that have higher transmissibility and disease severity and spread more rapidly in communities, making it imperative to find potential drug targets and effective therapeutics. To expedite the search for compounds with antiviral activity, different computational drug-repurposing strategies have been applied to identify appropriate candidates against target viruses. High-throughput virtual screening (HTVS) campaign in a large chemical space was used to discover inhibitors against SARS-CoV-2 envelope protein (EPRO), a structural protein and the smallest (8-12 kDa) of the major SARS-CoV-2 proteins, as it appears to be a promising target for drug development. It is important in the pathogenicity of the virus and is highly conserved. A set of potential EPRO inhibitor candidates were identified and are being evaluated in *in vitro* studies.

Firstly, the effect of the compounds on host cell lines is tested using an MTT assay to measure cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. The maximum non-toxic dose of the compounds is determined and used to treat viruses. Model viruses or surrogates are used to derive similar properties of a target virus while limiting environmental and researcher exposure to potentially dangerous pathogens. Here Murine hepatitis virus (MHV, strain A59), a beta-coronavirus that is considered a good model virus for SARS-CoV-2, is used, as it is evolutionarily and structurally very similar to SARS-CoV-2.

The effect of the selected compounds on the virus is evaluated at two levels, (i) cell-based assays to determine the reduction *in viral* infectivity upon treatment with the inhibitor. Viral infectivity is determined using the endpoint dilution assay (TCID) and the plaque assay (PFU). Different testing conditions, e.g., concentration of compound, time of incubation, are tackled in the experiments. Viral replication (ii) is followed by viral nucleic acid detection assays, e.g., qPCR, where the presence and amount of viral RNA is determined.

With *in vitro* analysis, we will confirm the efficacy of candidate EPRO inhibitors selected *in silico*. The best performing compounds will be good candidates for an antiviral drug to treat COVID-19 and other potential diseases caused by coronavirus.

P45

Screening of Lactic Acid Bacteria Strains for Proteolytic Activity

Botond Süli[1], Erika Bujna[1], Firas Alarawi[1], Stefan Savo Micevic[1], Olivia Csernus[1], Anett Szecsi[1], László Friedrich[2], Quang D. Nguyen[1]

[1]Department of Bioengineering and Alcoholic Drink Technology, Institute of Food Science and Technology
[2]Deparment of Livestock and Food Preservation Technology, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences

nguyen.duc.quang@uni-mate.hu

Lactic acid bacteria (LAB) are one of the most significant groups of probiotic organisms, commonly used in fermented dairy products. These microorganisms can enhance lactose digestion, stimulate the immune system, and prevent and treat diarrhea. They are able to synthetise wide spectrum of proteinases to hydrolyse the dairy proteins improving the nutritional values. Based on the results of genomic analysis, along with exo-fashion proteinases, they also can secrete some endo-fashion proteinases that can cleave the long peptide chain onto oligopeptide fractions. In this study, screening of lactic acid bacteria strains for production of proteinase was focused.

Proteolytic activity of twelve commercially available *Lactobacillus* strains and one *Pediococcus* strain were screened on agar media. The activity of the strains was evaluated based on the thickness of the clarification zones. All investigated strains produced clarification zones which refers to their proteolytic activity, although their intensity was different. The *Lactobacillus delbrueckii* subsp. *bulgaricus* 397, the *L. acidophilus* N2, the *Lactobacillus* 2231T strains exhibited strongest proteolytic activities on the agar media. It was also observed that the pH had significant effect on the proteolytic activity. Most strains performed bigger clean zones in slightly acidic pH condition. The thickest cleanzone was measured in the case of *L. delbrueckii* subsp. *bulgaricus* 397 at pH 5. Strains *Lacticaseibacillus rhamnosus* B01725, *Latilactobacillus curvatus* 2775 and *Lactiplantibacillus plantarum* 2142 showed relatively weak proteolytic activity. *Pediococcus* strain also exhibited the average proteolytic activity. Despite these results are preliminary, they can serve very good bases for studying the proteolytic activities from probiotic bacteria especially endo-proteinase activity.

This work was supported by the New Széchényi Plant Project No. GINOP_PLUSZ-2.1.1-21-2022-00048, by the NKFIH Project No. TKP2021-NVA-22. Süli, Alarawi and Savo are PhD students at Doctoral School of Food Science and got the fellowship from Stipendium Hungaricum Program.

P46

Effectiveness of Essential Oils as Inhibitors of Quorum Sensing Activity

Radovan Čobanović[1], Ivana Čabarkapa[2], Bojana Kokić[2], Slađana Rakita[2], Zorica Tomičić[2]

[1]*SP Laboratorija a.d. Bečej, Serbia*
[2]*Institute of Food Technology, University of Novi Sad, Serbia*

radovan.cobanovic@splaboratorija.rs

Quorum sensing (QS) represents a specific way of bacterial intercellular communication, which is enabled owing to their ability to detect and respond to cell population density by gene regulation. QS mechanisms are responsible for controlling the pathogenesis, virulence luminescence, motility, sporulation, and biofilm formation of many organisms by regulating gene expression. Therefore, research in this field is an attractive target for developing new natural antibacterial agents. Considering the importance of QS during bacterial pathogenesis, this research has been focused on the evaluation of the anti-QS properties of four essential oils (EOs) *Origanum heracleoticum*, *Origanum vulgare*, *Thymus vulgare*, and *Thymus serpyllum*, using biomonitor strain *Chromobacterium violaceum* CV026. Tests were conducted on Luria Bertani agar supplemented with N hexanol DL homoserine lactone (HHL) 10µl/50ml of agar. The anti-QS potential of the EOs was assayed in a range of 200 – 0.39 µl/ml using the disc diffusion method. EOs of *T. vulgaris* and *T. serpyllum* exhibited anti-QS activity indicated by a non-pigmented ring in a dilution-dependent manner. The lowest dilution of *T. vulgaris* and *T. serpyllum* in which they exhibited visually detectable inhibition of violacein synthesis was 6.25 µl/ml for both tested EOs.
O. heracleoticum and *O. vulgare* displayed different active principles, i.e. antimicrobial activity indicated by the inner clear ring and anti-QS activity indicated by the outer non-pigmented ring, in a concentration-dependent manner. The lowest dilution of *O. heracleoticum* and *O. vulgare* which exhibited visually detectable inhibition of violacein synthesis was 1.56 and 3.25 µl/ml, respectively. The main constituents of the tested EOs are monoterpenes (carvacrol, thymol, γ-terpinene, and p-cymene) and anti-QS properties of tested EOs can be mainly attributed to their activity. In particular, from the scientific literature, carvacrol and thymol show a sub-inhibitory effect against foodborne pathogens. Previous studies indicated that sub-lethal concentrations of carvacrol reduced the mobility of bacteria due to the ability of interference using the QS mechanism between the bacterial cells, thereby reducing the ability of biofilm formation. The precise mechanism by which carvacrol inhibits biofilm formation is still not fully understood.
The results imply that EOs represent a promising alternative for effective control of the emergence and spread of resistant pathogens.

P47

Presence of RNA Viruses in Raspberries

Radovan Čobanović[1], Katica Mihajlović[1], Bojana Kokić[2]

[1]*SP Laboratory A.D., Serbia*
[2]*Institute of food technology in Novi Sad, University of Novi Sad, Serbia*

radovan.cobanovic@splaboratorija.rs

Serbia is one of the largest exporter of berries in EU. Berries are a perishable food which can be consumed as fresh or minimally-processed as well as a frozen ingredient added to many foods. According to EFSA, contamination by Noroviruses and Hepatitis A viruses may occure at various stages of berry production chain.

Based on above mentioned, the aim of this study was to evaluate virological safety of raspberries (2057 samples) grown in Serbia. Analyzed samples were collected from various independent producers from January 2019 untill January 2022. Samples were analyzed on Norovirus (NoV) genogroups I (GI) and II (GII) and Hepatitis A (HAV). The applied method was based on ISO 15216-2:2019 Microbiology of the food chain — Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR — Part 2: Method for detection. Out of 2057 analyzed samples only 8 (0,4%) showed unsatisfactory positive result concerning NoV genogroup I (GI) and 1 (0,05%) concerning NoV genogroup II (GII) but neither one showed unsatisfactory positive result concerning HAV viruses.

As far as obtained results are concerned it can be concluded that Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) are properly implemented throughout berry production chain in Serbia. Due to high risk of contamination during berry production it is necessary to conduct permanent monitoring in order to provide microbiologically safe products that are exported to the EU.

P48

Tracking Sources of Contamination by *Listeria monocytogenes* in Retailed Cheeses Using Genome Sequencing

Renata Karpíšková, Kristýna Brodíková, Ivana Koláčková

Institute of Public Health, Medical Faculty, Masaryk University Brno, Czech Republic

renata.karpiskova@med.muni.cz

Listeria monocytogenes (LM) is a major foodborne pathogen causing listeriosis, an invasive disease with a high mortality rate in a vulnerable population. Listeriosis may cause epidemics but mainly occurs as sporadic cases in which the vehicle of infection remains unrecognized. Ready-to-eat foods of animal origin, raw fruits, and vegetables are known to be the main vehicles of infection. As the growth and persistence of LM depend on its ability to adapt to food processing environmental conditions, a fundamental challenge in food safety management thus remains hygiene.

In this study, we focus on the analysis of LM in samples of steamed cheeses.

The aims of this study are: i) to obtain LM isolates from cheeses obtained in the retail market, ii) to perform LM genotype characterization, and iii) to define the occurrence of persistent LM strains in the food business operator's environment.

Between 2014 and 2022, we purchased and examined 84 samples of packed cheeses from 19 manufacturers. We used EN ISO 11 290-1 and 2 for the detection, enumeration, and identification of LM.

Serotyping was performed by the slide agglutination method, using commercially available antisera (Denka Seiken, Japan), and subsequently confirmed by mPCR (Doumith et al., 2004). Genomic DNA was extracted using the Blood and Tissue kit (Qiagen, Germany), preparation of DNA libraries was performed by Nextera XT DNA Library Preparation Kit (Illumina) and sequencing on the Illumina platform was carried out externally using NextSeq sequencer. The raw sequence data were assembled *de novo* using the SKESA assembler version 2.4.0 integrated with Ridom SeqSphere+ software (version 8.3.0; Ridom GmbH, Germany) using downsampling to coverage 40x. Core genome (cg) MLST analysis was performed in Ridom SeqSphere+.

A total of 84 samples of steamed cheeses samples collected from retail in the Czech Republic in the 8-year period and were analyzed for the presence of LM. The pathogen was detected in 23 samples, out of 19 producers only 3 of them were detected positive for the presence of LM. None of the samples exceeded the number of 100 CFU/g at the end of shelf life. In products from producer A, LM of serotype 1/2a ST101 (MLST), in producer B, serotype 1/2b ST3, and in producer C, serotype 4b ST2 were detected.

The high homogeneity of LM genomes was found in each of the specific producers. Thus confirming the occurrence of persistent LM strains in the environment of these three food business operators.

P49

Utilizing Ccw12 as an Effective Anchor for Yeast Surface Display of Diverse Recombinant Proteins

Tea Martinić Cezar, Mateja Lozančić, Bojan Žunar, Vladimir Mrša, Renata Teparić

Faculty of Food Technology and Biotechnology, University of Zagreb

rteparic@pbf.hr

Yeast surface display is an innovative technique that can improve the stability and activity of recombinant enzymes by attaching the enzymes to the yeast cell wall. In this study, we focused on the C-terminal immobilization of recombinant proteins, as certain proteins require exclusive immobilization through their C-terminus to prevent loss of activity. C-terminal immobilization via the anchor Ccw12 protein has been a promising approach for the efficient display of various industrially relevant recombinant proteins due to the high abundance and stable attachment of the Ccw12 protein in the yeast cell wall. To further enhance the efficiency of C-terminal immobilization, we conducted a study where we examined a series of strains in which genes associated with the regulation of transcription of genes related to wall structure maintenance, mRNA processing, translation, quality control, and maintenance of ER morphology were deleted. We investigated the activity of the reporter Ccw12BLA protein in these mutants and compared it to the wild-type strain. Increased Ccw12BLA activity was observed in mutants lacking protein involved in amino acid deprivation response (gcn2), translational repressor for cell wall proteins (ssd1), a protein involved in endoplasmic reticulum-associated protein degradation pathway (doa10), and covalently linked proteins of the cell wall (scw4pir1pir2pir3pir4). Additionally, Ccw12BLA optimization was applied to the surface display of sucrose phosphorylase (SP), an enzyme that plays a role in glucosyl glycerol synthesis and has extensive applications in various industries including cosmetics, food, and pharmaceuticals. Sucrose phosphorylase (kindly provided by Prof. B. Nidetzky, TU Graz) was successfully expressed and immobilized through the Ccw12 anchor protein on the yeast cell wall in *gcn2, doa10, ssd1*, and *scw4pir1pir2pir3pir4* mutants, and its binding was confirmed through Western blot analysis. The activity measurement of Ccw12SP in these mutants showed a similar trend as observed for the Ccw12BLA recombinant protein, suggesting that these mutants are promising candidates for glucosyl glycerol production using yeast surface display technology. Also, we expressed methionine adenosyltransferase, a key enzyme in S-adenosylmethionine production, and confirmed its localization on the yeast cell wall.

P50

Comparison of *in Vitro* Methods to Assess the Antimicrobial Effects of Various Probiotics Against Common Clinical Pathogens

Sabina Fijan, Primož Kocbek, Maja Strauss

Faculty of Health Sciences, Institute for Health and Nutrition, University of Maribor, Maribor, Slovenia

sabina.fijan@um.si

Probiotics as microorganisms, administered in adequate amounts, are not only beneficial to host by improving and modulating the gut microbiota, but also beyond the gut, via the axes: gut–brain, gut–lungs, gut–skin. The skin is the largest organ in the human body and is colonized by a diverse microbiota that works in harmony to protect the skin. However, when skin damage occurs, the skin microbiota is also disrupted, and pathogens can invade the wound and cause infection. Probiotics or other beneficial microbes and their metabolites are one possible alternative treatment for combating skin pathogens via their antimicrobial effectiveness.

We investigated seven multi-strain dietary supplements with probiotics (OMNi-BiOTiC® HETOX, OMNi-BiOTiC® Stress repair, OMNi-BiOTiC® 6, OMNi-BiOTiC® Flora plus+, OMNi-BiOTiC® Active, NutriVital Ultra SB and Bio-Kult®) and eleven single-strain microbes (*Lacticaseibacillus rhamnosus* LGG, *Lacticaseibacillus paracasei* Shirota, *Limosilactobacillus reuteri* DSM 17938, *Lactiplantibacillus plantarum* DSM 2601, *Bifidobacterium animalis* subsp. *lactis* HN019, *Bifidobacterium animalis* subsp. *lactis* BB-12, *Bacillus coagulans* MTCC 5260, *Propionibacterium freudenreichii* DSM 20271, *Acidipropionibacterium acidipropionici* DSM 20272, *Propionibacterium freudenreichii* subsp. *Shermanii*, *Saccharomyces cerevisiae* var. *boulardii*) against 15 clinical wound pathogens using the agar spot assay, co-culturing assay, and agar well diffusion assay. We also conducted genera-specific and species-specific molecular methods to detect DNA in the dietary supplements and single-strain beneficial microbes.

We found that the multi-strain dietary supplements exhibited a statistically significant higher antagonistic effect against the challenge wound pathogens than the single-strain microbes and that lactobacilli-containing dietary supplements and single-strain microbes were significantly more efficient than the investigated *propionibacteria* and *bacilli*. Differences in results between methods were also observed, possibly due to different mechanisms of action. Individual pathogens were susceptible to different dietary supplements or single-strain microbes.

Perhaps an individual approach such as a 'probiogram' could be a possibility in the future as a method to find the most efficient targeted probiotic strains, cell-free supernatants, or neutralized cell-free supernatants that exhibit the highest antagonistic effect against individual clinical wound pathogens.

P51

Applicability of Propidium Monoazide for Discrimination Between Living and Dead Cells in Biofilms of *Candida albicans* and *Staphylococcus aureus*

Samuel Kendra, Barbora Gaálová-Radochová, Larysa Bugyna, Helena Bujdáková

Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia

kendra4@uniba.sk

The most used methods for quantification of living cells in the biofilm are cultivation (counting colony-forming units – CFU) or real-time PCR. However, these techniques are time-consuming and have limitations such as overestimation/underestimation of real cell number. The propidium monoazide qPCR (PMA-qPCR) can distinguish between living and dead cells in a sample with high accuracy due to the selective penetration of the dye (PMA) through the damaged membranes of dead cells and subsequent blocking DNA amplification. The aim of this work was to optimize PMA-qPCR for quantification of living and dead cells in 48 h biofilms formed by two clinically relevant pathogens – Candida albicans and Staphylococcus aureus. At first, specific genes and PCR primers for detection of respective bacteria/yeasts were selected (the *NUC* and *ACT1* genes for *S. aureus* and *C. albicans*, respectively). Afterwards, multiplex PCR was performed to verify and adjust melting temperatures (T_m) for each of the primers. The final T_m was set to 55 °C. For the photoactivation step required for PMA to covalently attach to the DNA, the PMA-LiteTM LED Photolysis Device was used. Different concentrations of PMA (25 and 50 μM) during incubation with the biofilm cells were tested. After live and dead cell control dCt determination, a final concentration of 25 μM PMA proved to be optimal. Finally, according to the obtained C_t values for each sample from PMA-qPCR (live and dead biofilm cells; with and w/o PMA), the percentage of viable cells was calculated and compared to CFU/mL. According to the preliminary data, 88% of viable cells were calculated in the single biofilm of *S. aureus* (corresponding to $4x10^6$ cells/mL); and 98% in the single biofilm of *C. albicans* (corresponding to $5x10^7$ cells/mL). The use of a molecular method for determining the number of DNA copies of surviving cells using PMA-qPCR seems to be a suitable alternative to the cultivation method. However, for the implementation of PMA-qPCR to practice, it is necessary to correctly set the conditions of the experiment, which are different for each type of microorganism. Such optimization is particularly important for mixed biofilms, which is the next goal of our study.

Acknowledgment. This work was supported by the Slovak Research and Development Agency under contracts of APVV-21-0302 and APVV-18-0075. This work was also supported by the EU Grant number 952398—CEMBO, Call: H2020-WIDESPREAD-05-2020—Twinning.

P52

The Possibility of Using the *Beauveria bassiana* for the Biological Control of Oak Lace Bug

Sanja Novak Agbaba[1], Nevenka Ćelepirović[1], Darko Pleskalt[2]

[1]*Croatian Forest Research Institute*
[2]*Croatian Forests d.o.o.*

sanjan@sumins.hr

Beauveria bassiana is an entomopathogenic fungus. It is used to control pests such as whitefly and trips, and spider mites on vegetables, fruit crops, and ornamentals. It is mainly used in agriculture in glasshouses. *B. bassina* is also reported for use in biological control against other pests such as aphids, caterpillars, leafhoppers, and hazelnut weevil but the experience is limited. The oak lace bug (*Corythucha arcuata, Heteroptera, Tingidae*) is an invasive forest pest that damages oak leaves by sucking sap. The damage leads to a decrease in photosynthetic activity and leaves drying. The consequence of the damage is the physiological weakening of the oaks. The aim of this paper is to investigate the application of the fungus *B. bassiana* on the mortality of the oak lace bug in laboratory conditions and in the field. In the laboratory experiment, the oak lace bug, collected from the pedunculate oak forest in Jastrebarsko, was sprayed with bioinsecticide Naturalis–L (based on the fungus *B. bassiana*) and with fungus isolated from naturally infected *C. arcuata*. In the field experiment the leaves of an oak tree infected with oak lace bugs were sprayed with the same bioinsecticide. After 5 and 7 days, the samples were analyzed, and dead, infected, and live bugs were recorded. DNA barcoding was used for the identification of fungal and insect specimens. The results showed a positive effect of spraying with Naturalis-L and natural autochthonous fungus on mortality and infection of oak lace bugs. Research on the use of *B. bassiana* and natural entomopathogenic fungus should be continued.

P53

Effect of Supplemented Nitrogen Source on Fermentation of Apple Juice by Probiotic Lactic Acid Bacteria

Sofia Radja Ziane[1], Erika Bujna[1], Sun Weizhe[1], László Friedrich[2], Quang D. Nguyen[1]

[1]*Department of Bioengineering and Alcoholic Drink Technology, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences*
[2]*Deparment of Livestock and Food Preservation Technology, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences*

EGPL83@uni-mate.hu

Probiotics are live microorganisms that confer beneficial effects on the host when administered in adequate amounts. Main probiotic food products are dairy based that may have limitations for some consumer groups such as lactose intolerants, or milk protein allergists etc. Fruit juices have been considered as suitable matrices for carrying probiotics, because they already contain many beneficial nutrients; have flavour profiles considered enjoyable by people of all ages, are considered healthy and refreshing drinks, and are consumed regularly. Nevertheless, fermentation of fruit juice with probiotic lactic bacteria results some technological errors especially in the sensorial properties such as odour taste, hospital taste etc. that make these types of products less acceptable for consumers. No doubt that the quality and quantity of nitrogen sources in fruit juice play an important role in the formation of aromatic compounds, thus in this study, the effects of supplemented nitrogen sources on the fermentation of apple juice by probiotic lactic acid bacteria were investigated.

Golden delicious apple juice, two probiotic bacteria *Lactiplantibacillus plantarum* 299v and *Lactobacillus acidophilus* La5 strains, two exogenous nitrogen sources soy and casein peptones were used in this study. The fermentation process was followed by determination of changes of pH, bacterial cells, antioxidant capacities. The results showed that both probiotic strains grew well in the apple juice with or without supplement of 2% nitrogen sources, but the *Lp. plantarum* 299v strain exhibited better growth dynamic than *L. acidophilus* La5 strain. Additionally, highest cell density 1.2×10^9 CFU/ml and pH 3.93 were observed, when fermentation of apple juice supplemented with 2% soy peptone by *Lp. plantarum* 299v strain for 24 hours. In the case of the antioxidant capacity, highest FRAP values were detected in apple juices supplemented with soy peptone. These values were 3.58 mM $FeSO_4$ and 3.64 mM $FeSO_4$ for *Lp. plantarum* 299v and *L. acidophilus* La5, respectively. The fermented apple juices smelled very freshly and pleasantly.

These results are preliminary, but very good for the further studies that are going on in our lab.

This work was supported by the New Széchenyi Plant Project No. EFOP-3.6.3.-VEKOP-16-2017-00005, by the NKFIH Project No. TKP2021-NVA-22. Ziane and Weizhe are PhD students at Doctoral School of Food Science and got the fellowship from Stipendium Hungaricum Program.

P54

Identifying Markers of Cattle Fecal Pollution Using Comparative Analysis of the 16S rRNA Gene

Tanja Žlender[1], Lucija Brezočnik[2], Vili Podgorelec[2], Maja Rupnik[1,3]

[1]*National laboratory of health, environment and food, Centre for Medical Microbiology, Department for Microbiological Research, Maribor, Slovenia*
[2]*Institute of Informatics, Faculty of Electrical Engineering and Computer Science, University of Maribor, Maribor, Slovenia*
[3]*Faculty of Medicine, University of Maribor, Maribor, Slovenia*

tanja.zlender@nlzoh.si

Cattle industry is one of the most important subsectors of agriculture that produces vast amounts of fecal waste. Cattle manure is commonly used as a bio-fertilizer and this can introduce fecally transmitted pathogens to the fields. The pollution can spread from agricultural fields to lakes, rivers, oceans, and groundwater. This can represent a risk for humans and animals, as well as environmental health. For successful remediation and identification of problematic agricultural areas, we need to identify the source of pollution. The aim of this study is to identify bacterial DNA sequences, specific to or highly associated with cattle feces and manure, that could potentially be used as Microbial Source Tracking (MST) markers.

Fecal samples (n = 430) from 49 species of wild and domestic animals including cattle and 5 cattle manure samples were collected in Slovenia between December 2020 and January 2022. The samples were subjected to 16*S* rRNA gene (V3-V4 hypervariable region) sequencing and analysis of zero-radius operational taxonomic units (ZOTU). Bacterial composition of different sample types was compared using NMDS and statistically confirmed using pairwise-PERMANOVA. In search for cattle-associated sequences, each ZOTU was split into shorter K-mers, shifting one base at a time. K-mers found in feces of different animal hosts were compared to the K-mers found in feces of cattle and manure to find sequences suitable for primer design in the field of MST.

We found that the bacterial composition of cow feces and manure is statistically different from feces of other domesticated and wild animals (pairwise PERMANOVA; $p < 0{,}05$). Using k-mer analysis, we identified multiple regions of interest suitable for cattle-specific primer design. These regions of DNA could potentially be used as MST markers for identifying fecal contamination deriving from cattle feces and manure.

P55

Oral Biofilms: A Source of Future Probiotics

Tomaž Rijavec[1], Urša Lovše[1], Teja Colja[1], Dmitrij Drev[1], Nina Katarina Grilc[2], Špela Zupančič[2], Julijana Kristl[2], Aleš Lapanje[1]

[1]*Jožef Stefan Institute, Slovenia*
[2]*Faculty of Pharmacy, University of Ljubljana, Slovenia*

tomaz.rijavec@ijs.si

Oral biofilms of healthy individuals are a source of beneficial bacterial strains that produce antimicrobial compounds, prevent the attachment of pathogens or compete with the pathogens for the same niches. In disease states, re-application of such bacteria back to the oral surfaces can be used in combination with other interventions, e.g. 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 (up to 500 strains) of aerobic, facultative and anaerobic strains, 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 [1,2], which enable us to combine together different bacterial strains into small communities as well as help them efficiently populate target surfaces. We discuss the importance of delivering a larger number of probiotic strains simultaneously and see the engineering of artificial communities as a superior approach to re-establishing a healthy oral microbiome [3].

References:

[1] Zupančič et al. 2018. Nanofibers with incorporated autochthonous bacteria as potential probiotics for local treatment of periodontal disease. Biomacromolecules, 19(11), pp.4299-4306.
[2] Mirtič et al. 2018. Development of probiotic-loaded microcapsules for local delivery: Physical properties, cell release and growth. European Journal of Pharmaceutical Sciences, 121, pp.178-187.
[3] Grilc et al. 2023. Nanofibers with genotyped Bacillus strains exhibiting antibacterial and immunomodulatory activity. J. Control Release, 10(355), 371-384.

P56
Human Gut Sporobiota – Optimization of DNA Isolation

Urša Miklavčič[1], Sandra Janežič[1,2], Maja Rupnik[1,2]

[1]National Laboratory for Health, Environment and Food (NLZOH), Centre for Medical Microbiology, Department for Microbiological Research, Maribor, Slovenia
[2]Faculty of Medicine, University of Maribor, Maribor, Slovenia

ursa.miklavcic@nlzoh.si

At least half of the microbes in the human gut can form endospores. Spores are resistant cell structures that can withstand many different environmental pressures, and as such, they represent the first colonizers after antibiotic treatment, inflammation, and disease in the human gut. The ability of spores to survive unfavourable conditions in and outside of the gut allows transfer between hosts and/or environments. Although two well-known procedures are available for spore selection in cultivation procedures (exposure to high temperatures or to ethanol), they do not give optimal results when preparing sporogenic fraction for NGS analysis. In this study, we aimed to compare different approaches for the isolation of DNA from sporulated fraction from faecal samples. After ethanol and heat shock, spores were either washed with water or treated with ethidium monoazide (EMA). EMA binds to free DNA and DNA in damaged cells, leaving DNA within spores intact. DNA from treated and untreated faecal samples was isolated with MasterPure DNA and RNA purification kit with added bead-beating step. To compare the effect of different combinations of treatments we amplified and sequenced V3-V4 region of 16S rRNA gene. The sequences from samples treated with heat shock and only washed with water clustered with the samples of the entire microbiota, while the samples additionally treated with EMA clustered separately. For the prevalent phyla, we compared relative phylum abundance in each treatment with matched phylum in microbiota samples. We showed that the relative abundance of phyla without known spore-formers *Bacteroides* and *Proteobacteria* decreases drastically, while phyla with a few spore-formers such as *Actinobacteria* the decrease in relative abundance is smaller. The relative abundance of ZOTUs (zero-radius operational taxonomic unit) from the phylum with the most known spore-forming bacteria *Firmicutes* was increased. This is most apparent in samples treated with ethanol shock and irradiation with bright light for 10 minutes after incubation with EMA. Thus, with the optimized protocol for the isolation of DNA spore-forming bacteria from the human gut, we increased the amount of sequences from spore-formers.

P57

Screening of Mutual Interactions of Intestinal Microbiota and Cemtirestat in Rat Models of Diabetes

Zuzana Farkas[1], Jelena Pavlović[1], Magdaléna Rusková[1], Marek Lepáček[2], Nikola Klištincová[1], Francesca Maisto[1], Mária Bučková[1], Andrea Puškárová[1], Marta Šoltésová Prnová[2], Milan Štefek[2], Domenico Pangallo[1]

[1]*Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia*
[2]*Center of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia*

zuzana.farkas@savba.sk

Microbial imbalance (dysbiosis) in the gastrointestinal tract can be associated with metabolic disorders such as obesity, insulin resistance, diabetes, and immune system disorders [1]. Currently, extensive research is underway to understand these interactions. One of the main goals of our research is to investigate the interactions between the drug cemtirestat (3-Mercapto-5H-1,2,4-Triazino[5,6-b]Indole-5-Acetic Acid) [2] and the intestinal microbiota in diabetes, and their possible effects on the development of diabetic peripheral neuropathy in type 1 and type 2 diabetes rat models. Specifically, by Oxford Nanopore Technology (ONT) sequencing using MinION device, a deeper insight into the microbial structure and species diversity of the intestinal flora in rat models was provided. The optimized experimental and bioinformatic pipeline to perform the whole genome metagenomic study of the rat gut microbiome was proposed in this work. The data preprocessing included basecalling, quality control, adapter trimming and filtering, and metagenome assembly steps. The assembled contigs were subjected to downstream analysis of the taxonomic classification by Kraken2 (https://ccb.jhu.edu/software/kraken2/) which was followed by computing the relative abundance of different species in the samples, estimation of the alpha and beta-diversities by calculating several metrics using phyloseq (1.28.0) package (R version 3.6.1). The obtained results of the microbial composition profiles between diabetic rats treated with cemtirestat and untreated diabetic rats revealed similar diversities within the individual rats. Our study demonstrates that metagenomic sequencing by MinION can bring more knowledge on the influence of the investigated drug on the gut microbiome.

This study was supported by project of APVV-20-0411 “Gut microbiota and diabetic peripheral neuropathy: effect of cemtirestat in rat models of diabetes” and by the European Regional Development Fund project: 313011V578.

[1] Sittipo, P., Lobionda, S., Lee, Y.K. et al. Intestinal microbiota and the immune system in metabolic diseases. J Microbiol. 56, 154–162 (2018).

[2] Soltesova Prnova, M., Svik, K., Bezek, S. et al. 3-Mercapto-5H-1,2,4-Triazino[5,6-b]Indole-5-Acetic Acid (Cemtirestat) Alleviates Symptoms of Peripheral Diabetic Neuropathy in Zucker Diabetic Fatty (ZDF) Rats: A Role of Aldose Reductase. Neurochem Res 44, 1056–1064 (2019).

TRIBUNE LECTURES

TL1

The Potential of Microencapsulation in Cheese Production

Nataša Mikulec, Jasminka Špoljarić

Faculty of Agriculture, University of Zagreb, Croatia

nmikulec@agr.hr

The aim of the project "Potential of microencapsulation in cheese production" was to investigate the possibility of microencapsulation of natural rennet obtained from the rennet of lambs (which are mostly not further used) and the possibility of simultaneous microencapsulation of rennet and isolated and identified microbial culture (lactic acid bacteria). This research has revitalised the production technology of rennet for the production of cheese “Paški sir” by introducing a new and more modern method of microencapsulation of rennet and microbial culture.

The applied method of rennet and microbial culture production eliminates the use of imported industrial rennet and milk cultures, which is of particular importance for protecting the originality of the cheese according to EU criteria. The result of the research refers to the application of a traditional technological process modified in the laboratory in the production technology of different types of cheese, regardless of the type of milk. The isolation of autochthonous microbial cultures and their use in cheese production contribute to the preservation of biodiversity and to a greater diversity of cheese varieties on a global and regional level. The technology developed allows the product to be adapted to the needs of customers in the production of traditional and "boutique" cheeses.

TL2

Prevention of Late Blowing in Cheese

Johanna Burtscher, Konrad Domig

University of Natural Resources and Life Sciences, Vienna, Austria

johanna.burtscher@boku.ac.at

Quality defects in hard and semi-hard cheeses are a major concern for the dairy industry. A particularly severe defect, known as late blowing, is caused by gas-producing bacteria, particularly clostridia and propionibacteria. The metabolism of these two bacterial genera produces large amounts of carbon dioxide gas and/or hydrogen gas, resulting in unwanted holes and cracks in the cheese. In addition, rancid butyric acid or nutty propionic acid is formed, resulting in intense off-flavors. Late blowing does not pose a health risk to consumers, but cheese producers face significant financial losses due to the reduced quality of their product. Therefore, the prevention of blowing defects is crucial. Several approaches have been established during cheese production to prevent late blowing, including (1) control of production parameters, such as salt concentration or ripening temperatures, (2) addition of substances that inhibit germination and outgrowth of clostridia, such as nitrate, lysozyme, bacteriocins or bacteriocin-producing starter cultures, and (3) removal of spoilage bacteria from raw milk, such as pasteurization to inactivate propionbacteria or bactofugation or microfiltration to reduce clostridial endospores in milk. The effectiveness of these technological measures or additives depends strongly on low initial bacterial counts. Therefore, high-quality hard cheese production starts with measures to prevent bacterial contamination during primary raw milk production, such as feeding, barn and milking hygiene. However, the quantification and differentiation of gas-producing bacteria in milk remains challenging and is a subject of ongoing research. The implementation of a recently developed method for the quantification of spores of butyric acid producing clostridia in raw milk by several dairies has shown that monitoring clostridial spores helps to prevent late blowing in cheese. It also underscores the role of appropriate analytical methods to assess raw milk quality as an essential part of cheese quality assurance.

TL3

Influence of Maceration Time and Temperature on Bioactive Compounds and Antioxidant Capacity in Malvazija Istarska and Teran Wines

Sanja Radeka, Sara Rossi, Ena Bestulić, Fumica Orbanić

Institute of Agriculture and Tourism, Poreč, Croatia

sanja@iptpo.hr

The aim of this study was to explore the effect of applying maceration processes of different durations and temperatures on phenolic compounds concentration and antioxidant activity of Malvazija istarska and Teran wines. Vinification took place in vintages 2018-2019 at the Institute of Agriculture and Tourism (Poreč). Two days prefermentative mash cooling of Malvazija istarska (at 8°C) and heating treatment (at 45°C) of Teran, maceration during fermentation and different post-fermentative prolonged maceration treatments were studied and compared to control treatment (7 days maceration for Teran and non-maceration treatment for Malvazija istarska). Both, Malvazija istarska and Teran treatments were inoculated with 30 g/hL of selected yeast Saccharomyces cerevisiae (Malvazija istarska with Fermol Arome Plus and Teran with Fermol Mediterranée, AEB). Total phenolic content was determined by UV-Vis spectroscopy. The analysis of individual phenolic compounds was carried out by HPLC. The antioxidant capacity of the wines was determined by FRAP and ORAC assay. Statistical data analysis was performed using one-way ANOVA and LSD test. The total polyphenolic content in Malvazija istarska wines varied from 139.88 to 340.33 mg GAE/L and in Teran wines from 2448.33 to 2916.67 mg GAE/L. The highest total phenolic content in Malvazija istarska wines was observed in post-fermentative maceration treatments of 14 and 42 days, while the highest increase in Teran wines was noted in treatments produced by prefermentative heating and postfermentative prolonged maceration treatment of 21 days. Individual phenolic compounds obtained a similar trend as total phenolic content when observing both investigated variety wines. In Malvazija istarska wines antioxidant capacity increased proportionally with the maceration duration, with control wine having the lowest and 42 days treatment wine the highest values. However, in Teran wines, the highest influence on the antioxidant capacity was observed in wine produced by prefermentative heating. With applying different vinification techniques, significant differences were obtained between treatments of both varieties, even though there were no differences in yeast strain between treatments of each variety. The results obtained in this study may serve producers in choosing the adequate technological practice for improving wine quality and its bioactive properties. The study was funded by the HRZZ under the project VINUM SANUM (IP 2018-5049).

TL4

Influence of Non-*Saccharomyces* Yeasts on Chemical and Sensory Characteristics of Malvazija Istarska White Wine with the Emphasis on Volatile Esters as Revealed by Comprehensive Two-Dimensional Gas Chromatography

Doris Delač Salopek[1], Ivana Horvat[1], Sanja Radeka[1], Tomislav Plavša[1], Ana Hranilović[2,3], Silvia Carlin[4], Urska Vrhovsek[4], Igor Lukić[1,5]

[1]*Institute of Agriculture and Tourism, Croatia*
[2]*Department of Wine Science, The University of Adelaide, Australia*
[3]*Laffort, France*
[4]*Metabolomics Unit, Food and Nutrition Area, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Italy*
[5]*Centre of Excellence for Biodiversity and Molecular Plant Breeding, Croatia*

igor@iptpo.hr

Although yeasts from genus Saccharomyces are most commonly used in wine production, there is a growing interest in the use of non-*Saccharomyces* yeasts as fermentation starters because of their numerous beneficial features. Non-Saccharomyces yeasts have the ability to reduce the content of ethanol and thus mitigate negative impacts of climate change. Some non-*Saccharomyces* yeasts can modify acidity through natural deacidification or synthesis of relevant amounts of lactic acid. They can be used for bioprotection by limiting the development of potentially undesirable microbiota, while some can improve protein stability by affecting pathogenesis-related proteins. One of the most important applications of non-Saccharomyces yeast is their use for obtaining wines with distinct aroma profiles. In this experiment, grape must of Malvazija istarska was inoculated with five commercial non-*Saccharomyces* yeasts and a *Saccharomyces cerevisiae*/*Saccharomyces paradoxus* hybrid. *Saccharomyces cerevisiae* was inoculated at 2% (v/v) of ethanol to finish fermentations and as a monoculture control. Produced wines were tested for a variety of features, with the emphasis on volatile aroma compounds, especially esters, key compounds that determine wine aroma and flavour. More than 400 volatiles were determined by untargeted metabolomics using two-dimensional gas chromatography (GC×GC/TOF-MS) complemented by GC-MS, of which more than a hundred esters, providing one of the most detailed profiles of esters in wine. *Torulaspora delbrueckii* showed the highest potential to produce ethyl isobutyrate, isopropyl, isoamyl, 2-phenethyl and geranyl acetates, ethyl 3-hexenoate and ethyl 4-hexanoate. *Pichia kluyveri* produced most trans-3-hexenyl acetate, ethyl 2-methylbutyrate and ethyl 3-methylbutyrate, *Metschnikowia pulcherrima* produced the highest amounts of

ethyl decanoate and ethyl phenyl lactate, while *S. cerevisiae*/*paradoxus* hybrid increased the concentration of 2-phenethyl isobutyrate. *Lachancea thermotolerans* wine was the most abundant in isobutyl octanoate, while esters of isoamyl alcohol with various acids were increased by *Schizosaccharomyces pombe*. *S. pombe* proved its deacidification ability, while *L. thermotolerans* produced most lactic acid. The lowest total PR proteins level was found in *P. kluyveri* inoculated wines. The approach reported may have practical application in better understanding and managing the content and composition of Malvazija istarska and wine in general.

SPONSOR LECTURE

SL1
eDNA Detection: A Powerful Tool for Monitoring Microbes in the Environment

Lucija Markulin[1], Dora Pavić[2], Dorotea Grbin[2,3], Sandra Hudina[3], Anđela Miljanović[2], Rok Košir[4], Filip Varga[5,6], Josip Ćurko[2], Zoran Marčić[3], Ana Bielen[2], Uršula Prosenc Zmrzljak[4]

[1]*Labena d.o.o., eDNALabs, Croatia*
[2]*Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia*
[3]*Faculty of Science, University of Zagreb, Croatia*
[4]*Labena Ltd, BIA Separations CRO, Slovenia*
[5]*Faculty of Agriculture, University of Zagreb, Croatia*
[6]*Centre of Excellence for Biodiversity and Molecular Plant Breeding, CoE CroP-BioDiv, Croatia*

lucija.markulin@labena.hr

Saprolegnia parasitica is an oomycete pathogen that can have a significant impact on aquaculture operations. It primarily affects salmonids, including eggs and adult fish, but it can also infect a wide range of other aquatic organisms. *Saprolegnia* infections can cause mortality, reduced growth rates, and increased stress in fish and other aquatic organisms, resulting in decreased productivity and economic losses. Traditional methods of detection of *S. parasitica* are often carried out after a disease outbreak, and they can be labour-intensive and invasive, as they often require the capture and culling of host animals. However, the use of environmental DNA (eDNA) methods can help in monitoring *S. parasitica* more efficiently and effectively.

eDNA refers to the genetic material release into the environment by organisms. It can be obtained from water, sediment, soil, and other environmental sources. eDNA methods enable detection of presence or absence of a organisms of interest in the environment. There are two approaches for detecting species using eDNA: single-species detection using qPCR or ddPCR, and multi-species detection using DNA metabarcoding.

Here, we report the use of a single-species detection approach in development of a highly sensitive ddPCR assay for quantifying *S. parasitica* load in eDNA samples. New primers were designed targeting part of the ITS region that enables discrimination between *S. parasitica* and closely related species. The assay analysed pathogen load in eDNA extracted from water samples, detecting *S. parasitica* in 62 % of analysed samples. Additionally, swab analysis of trout with skin injuries showed significantly higher *S. parasitica* skin loads compared to

healthy specimens, with the pathogen detected in 91 % of swabs from injured fish, indicating its dominance as an opportunistic skin pathogen in trout.

In conclusion, the developed ddPCR assay can be utilized as a non-invasive monitoring approach for rapid and simple detection of *S. parasitica* in the environment. The use of eDNA methods, such as ddPCR, can provide a more efficient and effective approach for early detection and monitoring of *Saprolegnia* infections. By accurately predicting outbreaks through ddPCR-based monitoring, the use of toxic antioomycetic chemicals could be adjusted based on the actual pathogen load, potentially leading to more sustainable disease management practices in aquaculture operations.

LIST OF AUTHORS

A	
Abalymov, Anatolii A.	P2, P7
Alarawi, Firas	P45
Andreieva, Yuliia	OP6
Aserse, Aregu	OP11
B	
Babić, Ivana	OP9, OP10, P4, P6
Bagi, Zoltán	IL9, P15
Balázs, Dóra	OP15
Bandelj, Dunja	OP8
Banić, Martina	P23, P24
Barišić, Ivan	OP10
Beauvais, Valentin	P18
Bestulić, Ena	TL3
Bethala, Kumeera	P40
Bielen, Ana	SL1
Bilská, Katarína	OP13, P27
Bolanča, Tomislav	OP3, P31
Borovec, Petra	P21
Brezočnik, Lucija	P54
Brkljačić, Lidija	P17
Brodíková, Kristýna	P20, P48
Brunello, Andrea	OP16
Brutscher, Johanna	P41
Bugyna, Larysa	P51
Bujdák, Juraj	P25, P27
Bujdáková, Helena	OP13, P8, P25, P27, P51

..B	
Bujna, Erika	OP7, P45, P53
Bule Možar, Kristina	OP3, P31
Burtscher, Johanna	TL2
Butorac, Katarina	P23, P24
Bučková, Mária	OP12, P57
Bäck (Hirz), Melanie	IL7
Bär, Séverine	IL10
Bírošová, Lucia	P28, P30
C	
Carlin, Silvia	TL4
Ceilinger, Marijana	P23
Cheung, Sally	IL9, P15
Chrzanowski, Grzegorz	OP6
Civilini, Marcello	OP16
Colautti, Andrea	OP16
Colja, Teja	P55
Comi, Giuseppe	P34
Csernus, Olivia	P45
Cverenkárová, Klára	P30
Cvetnić, Matija	P31
Czatzkowska, Małgorzata	P36
Czucz Varga, Jarmila	OP13, P25
Č	
Čabarkapa, Ivana	P46
Čadež, Neža	IL8
Čobanović, Radovan	P46, P47
Čukelj, Danijela	P23

..Č	
Čuljak, Nina	P23, P24
Ć	
Ćelepirović, Nevenka	P42, P52
Ćurko, Josip	SL1
D	
Dadi, Nitin Chandra teja	OP13
Deev, Dmitrii	OP4, P2, P7, P13, P14
Dekić Rozman, Svjetlana	OP10
Delač Salopek, Doris	TL4
Dembińska, Katarzyna	P37
Dimkić, Ivica	OP8
Dmytruk, Kostyantyn	OP6
Dmytruk, Olena	OP6
Domig, Konrad	TL2, P41
Dreisewerd, Lennart	P44
Drev, Dmitrij	P55
Drk, Sara	P43
Dán, Kinga	P29
Dąbrowska, Grażyna B.	P37
Dželalija, Mia	P43
Đ	
Đermić, Damir	P11, P12
Đermić, Edyta	P11
F	
Fafanđel, Maja	OP9, P4
Farkas, Attila	IL9, OP2
Farkas, Zuzana	OP12, P57
Fayura, Liubov	OP6

..F	
Fedorovych, Daria	OP6
Felice, Antonella	OP16
Feliciello, Isidoro	P11
Fijan, Sabina	P35, P50
Filep, Robert	OP7
Fičko, Karlo	P16
Flieder, Mathias	OP1
Franchi, Marinella	OP16
Friant, Sylvie	IL10
Friedrich, László	P45, P53
Furdek Turk, Martina	OP9, P4
G	
Gadpayale, Jagannath V.	P40
Galgóczy, László	P29, P32
Gaálová-Radochová, Barbora	P51
Gitura Kimani, Bernard	P40
Gligora Udovič, Marija	P5
Goreta Ban, Smiljana	P21
Grbin, Dorotea	SL1
Grilc, Nina Katarina	P55
Gupta, Vijai Kumar	OP7
H	
Harnisz, Monika	P10, P36
Harwood, Colin	IL6
Herchlová, Karin	P22
Hladnik, Matjaž	OP8
Hollá, Katarína	P28
Horvat, Ivana	TL4
Horvat, Milena	P3
Hosseini, Farzaneh	P14

..H	
Hranilović, Ana	TL4
Hudina, Sandra	SL1
Hulisz, Piotr	P1
Huđ, Anastazija	P6, P17
I	
Iacumin, Lucilla	OP16, P34
Ivanković, Siniša	P11
Ivančić Baće, Ivana	P19
J	
Janakiev, Tamara	OP8
Janežič, Sandra	P56
Jelić, Marko	OP10
Jelušić, Aleksandra	OP8
Jerić, Ivanka	P23
Jordao, Luisa	P8
Jukić, Marko	P44
K	
Kaczmarek-Szczepańska, Beata	P37
Kajić, Sanja	OP11, P21
Kalwasińska, Agnieszka	P1, P37
Kandeler, Ellen	P6
Karija Vlahović, Monika	P42
Karpiskova, Renata	P20
Karpouzas, Dimitrios	OP9, P4
Karpíšková, Renata	P48
Kele, Zoltán	P29, P32
Kendra, Samuel	P8, P51
Kerekes, Erika Beáta	P40
Kikhney, Judith	P8
Kilin, Akos	OP7

..K	
Kirkegaard, Rasmus	OP1
Kličtincová, Nikola	OP12, P57
Kobe, Nina	P44
Kocbek, Primož	P50
Kogovšek, Polona	P44
Kohari-Farkas, Csilla	OP7
Kokić, Bojana	P46, P47
Kolackova, Ivana	P20
Kolda, Anamarija	P5
Koláčková, Ivana	P48
Kos, Blaženka	P23, P24
Kosjek, Tina	OP4
Kourist, Robert	IL5, P26
Kovács, Kornél L.	IL9, P15
Kovács, Tamás	P40
Košak, Aljoša	P3
Košir, Rok	SL1
Kredics, László	OP15
Kristl, Julijana	P55
Kulaš, Antonija	P5
Kumar, Sweta Binod	P1, P37
Kursawe, Laura	P8
Kučić Grgić, Dajana	OP3, P31
L	
Lapanje, Aleš	OP4, P2, P3, P7, P13, P14, P55
Laszlovszki, Gabor	OP7
Lazarević, Boris	P17
Leboš Pavunc, Andreja	P23, P24
Lehmann, Martin	IL7

..L	
Lepáček, Marek	P57
Lindström, Kristina	OP11
Lobnik, Aleksandra	P3
Lorber, Mateja	P35
Lovše, Urša	P55
Loy, Alexander	OP1
Lozančić, Mateja	P49
Lukić, Igor	TL4
Lučić, Mavro	OP9, P4
Ł	
Łukowicz, Krzysztof	P38
M	
Mackuľak, Tomáš	P30
Mahnic, Aleksander	OP14
Maisto, Francesca	OP12, P57
Maravić, Ana	OP10
Marcsisák, Fanni	P29
Marhan, Sven	P6
Marik, Tamás	OP15
Marinšek Logar, Romana	P9
Markulin, Lucija	SL1
Maróti, Gergely	IL9, OP2, P15
Martin, Nicole H.	P33
Martinić Cezar, Tea	P49
Martinjak, Viktorija	P31
Marx, Hans	OP6
Marčić, Zoran	SL1
Matic, Ivan	IL2
Matijević, Slavica	OP9, P4

..M	
Matikhaye, Swapnil Bhimrao	P40
Mattanovich, Diethard	IL1, OP6
Mešić, Armin	P17
Micevic, Stefan Savo	P45
Michalska-Sionkowska, Marta	P38
Mihajlović, Katica	P47
Miklavčič, Urša	P56
Mikulec, Nataša	TL1
Miličević, Jelena	P23
Miljanović, Anđela	SL1
Miloloža, Martina	OP3, P31
Mičetić-Turk, Dušanka	P35
Mosrin-Huaman, Christine	P18
Moter, Annette	P8
Motyka, Olena	OP6
Mrkonjic Fuka, Mirna	P41
Mrša, Vladimir	P49
Myrtollari, Kamela	IL5
N	
Nguyen, Quang D.	OP7, P45, P53
Nigl, Andrea	IL5, P26
Novak Agbaba, Sanja	P42, P52
Novak, Jasna	P23, P24
Novačić, Ana	P18
O	
Ogrinc, Nives	OP4
Olejníková, Petra	IL11, P22, P28, P30
Orbanić, Fumica	TL3
Orsi, Renato H.	P33

..O	
Osyczka, Anna Maria	P38
P	
P. Allwin Mabes Raj, A. F.	P3
Palijan, Goran	P6, P16, P17
Pangallo, Domenico	OP12, P57
Pap, Bernadette	IL9, OP2
Papp, Rebeka	P32
Paukszto, Łukasz	P10
Pavić, Dora	SL1
Pavlović, Jelena	OP12, P57
Pejchalová, Marcela	P37
Pejić, Ivan	OP11
Pellegrini, Michela	P34
Penava, Lenkica	P23
Perdana, Muhammad Iqbal	P40
Petek, Blaž	P9
Petek, Marko	P6, P17
Petrić, Ines	OP9, P4, P5, P6, P17
Petschacher, Barbara	IL7
Pichler, Harald	IL7
Piernik, Agnieszka	P1
Pjevac, Petra	OP1
Plavša, Tomislav	TL4
Pleskalt, Darko	P52
Plešnik, Helena	OP4
Podgorelec, Vili	P54
Podlipnik, Črtomir	P44
Podrzaj, Lucija	P33, P41
Pole, Lucia	P5, P17

..P	
Poór, Péter	P32
Pribus, Marek	P27
Primc, Gregor	P13
Prosenc Zmrzljak, Uršula	SL1
Puljko, Ana	OP10, P43
Puškárová, Andrea	OP12, P57
R	
Radeka, Sanja	TL3, TL4
Radja Ziane, Sofia	P53
Radochová, Barbora	P8
Radovčić, Marin	P19
Rahmouni, A. Rachid	P18
Rajnović, Ivana	OP11, P17, P21
Rakita, Slađana	P46
Ramljak, Ana	OP9, P4
Ramteke, Pramod W.	P40
Rani, Vaishali	OP2
Rattei, Thomas	OP1
Recek, Nina	P13
Repar, Jelena	P12
Retelj, Matjaž	P39
Richert, Agnieszka	P37
Riezman, Howard	IL7
Rijavec, Tomaz	P3
Rijavec, Tomaž	OP4, P2, P13, P14, P55
Rinaldi, Bruno	IL10
Rolbiecki, Damian	P10
Rossi, Sara	TL3
Rozsnyói, Ákos	OP15

..R	
Ruchala, Justyna	OP6
Rupnik, Maja	OP14, P39, P54, P56
Rusková, Magdaléna	OP12, P57
Rákhely, Gábor	P29, P32
S	
Saccomanno, Nicola	OP16
Sawicki, Jakub	P10
Schneider, Olha	OP5
Segéňová, Ivana	P22
Senko, Helena	P5, P6, P17
Shetty, Prateek	IL9, OP2, P15
Shintani, Masaki (Masa)	IL12
Sibirny, Andriy	IL3, OP6
Sikora, Sanja	OP11, P21
Somkuwar, Subhash R.	P40
Souza, Cleiton Martins	IL7
Spasic, Jelena	P26
Stloukalová, Berenika	P26
Stolterfoht-Stock, Holly	IL7
Strauss, Maja	P35, P50
Strojnik, Lidija	OP4
Stuparević, Igor	P18
Swiontek Brzezinska, Maria	P1, P37
Szecsi, Anett	P45
Szekeres, András	OP15
Szuhaj, Márk	IL9, P15
Süli, Botond	P45
Š	
Šamec, Dunja	P17
..Š	
Šoltésová Prnová, Marta	P57
Špoljarić, Jasminka	TL1
Štefek, Milan	P57
Štefánek, Matúš	OP13
Štrok, Marko	P14
Šušković, Jagoda	P23, P24
T	
Taden, Andreas	IL5
Takó, Miklós	P40
Tanuwidjaja, Irina	P41
Teparić, Renata	P49
Terhes, Gabriella	OP2
Tocino-Márquez, Inmaculada	OP1
Tomičić, Zorica	P46
Toth, Nina	P21
Trmčić, Aljoša	P33
Tsyrulnyk, Andriy	OP6
Tyagi, Chetna	OP15
Tóth, Gábor K.	P29, P32
Tóth, Liliána	P29, P32
U	
Udiković Kolić, Nikolina	OP9, OP10, P4, P6, P17, P43
Ukić, Šime	OP3, P31
V	
Varga, Filip	SL1
Varga, Mónika	P40
Vasileiadis, Sotirios	OP9, P4
Vasiljevic, Branka	IL4
Vovko, Petra	P39

..V	
Vrhovsek, Urska	TL4
Vu, Vi N.H.	OP7
Vukićević, Slavoljub	OP8
Vágvölgyi, Csaba	OP15, P40
Váradi, Györgyi	P32
Víglaš, Ján	IL11
W	
Walczak, Maciej	P38
Warżyńska, Oliwia	P38
Weizhe, Sun	P53
Wiedmann, Martin	P33
Wirth, Roland	IL9, P15
Wriessnegger, Tamara	IL7

Z	
Zahradka, Davor	P11, P12
Zahradka, Ksenija	P12
Zehl, Martin	OP1, OP5
Zelenik, Katja	P39
Zotchev, Sergey B.	OP1, OP5
Zugan, Maja	OP4, P2, P14
Zupančič, Špela	P55
Zver, Mark	P13
Ž	
Žlender, Tanja	P39, P54
Žunar, Bojan	P18, P49
Žučko, Jurica	OP9, P4, P6

Organised by
Croatian Microbiological Society,
Czechoslovak Society for Microbiology,
Hungarian Society for Microbiology,
Slovenian Microbiological Society,
Turkish Society of Microbiology.

Supported by Croatian Academy of Engineering

Supported by Faculty of Food Technology and Biotechnology University of Zagreb

Supported by Federation of European Microbiological Societies

www.hmd-cms.hr/power2023