8th Croatian Congress of Microbiology

with International Participation



PROGRAMME AND

ABSTRACTS

22–25 SEPTEMBER 2026

TRAKOŠĆAN · CROATIA

8th Croatian Congress of Microbiology with International Participation

PROGRAMME AND ABSTRACTS

September 22 – 25, 2026
Trakošćan, Croatia

Publisher

Croatian Microbiological Society

Pierottijeva 6, HR-10000 Zagreb, Croatia

www.hmd-cms.hr

For publisher

Dijana Škorić

Editors

Ines Sviličić Petrić

Andreja Leboš Pavunc

Ana Bielen

Domagoj Kifer

ISBN 978-953-7778-22-4

Organised and hosted by

Croatian Microbiological Society

Co-organised by

University of Zagreb

Faculty of Food Technology and Biotechnology

Sponsored by

Biosistemi grupa

Biovit

IDEXX

INEL – medicinska tehnika

Sponsored by

KEFO

Kemolab

Medic

Micro+polo

Novogene

Vaxxinova

Organising Committee

Ines Sviličić Petrić, *president*

Andreja Leboš Pavunc, *secretary*

Marina Svetec Miklenić, treasurer

Ivana Babić

Martina Banić

Domagoj Kifer

Scientific Committee

Ana Bielen, *president*

Egon Bech Hansen (Denmark)

Sandra Hudina (Croatia)

Denis Kutnjak (Slovenia)

Jasna Novak (Croatia)

Giuseppe Spano (Italy)

Monika Šabić Runjavec (Croatia)

Maja Šegvić Klarić (Croatia)

Irena Tabain (Croatia)

Davor Zahradka (Croatia)

CONTENTS

PRESIDENTS' FOREWORD **7**

GENERAL INFORMATION **8**

PROGRAMME **9**

KEYNOTE LECTURES **16**

INVITED LECTURES **19**

SHORT ORAL PRESENTATIONS **29**

SPONSORED LECTURE **57**

CROSS-DISCIPLINARY MODULES **59**

WORKSHOPS **63**

POSTER PRESENTATIONS **66**

LIST OF AUTHORS **116**

PRESIDENTS' FOREWORD

Dear colleagues and friends,

Welcome to the 8th Croatian Congress of Microbiology with International Participation, held in Trakošćan from 22 to 25 September 2026. With its fairy-tale castle and wooded surroundings, Trakošćan offers a memorable setting to share our work, exchange ideas and spend time together.

Our theme, "Microbiology for a Sustainable Future", reflects the contribution of microbiology to human, animal and plant health, environmental resilience, food security and the sustainable bioeconomy. Through invited lectures, oral presentations and posters, we will explore microbial processes and interactions relevant to these areas.

A central aim of this Congress is to bring together microbiologists across different fields, sectors and career stages. By learning more about one another's work, we can strengthen collaboration, exchange ideas beyond our own specialities and make better use of our collective expertise. Our international colleagues further enrich this exchange and extend these connections beyond Croatia.

With this in mind, the programme also includes cross-disciplinary modules and workshops on artificial intelligence, publishing and science communication, and collaboration with industry. We especially encourage early-career colleagues to participate actively, share their ideas and help shape the future activities of the Society. And, as always, some of the best discussions may continue outside the lecture hall - during the excursion, gala dinner, over štrukli or at the microbiology pub quiz.

Our commitment to communication extends beyond the scientific community. After the Congress, "Microbes at Trakošćan", a public event for children, will invite young visitors to explore the microbial world through interactive activities.

We thank the Organising and Scientific Committees, invited speakers, organisers, authors, sponsors and everyone who contributed to the programme.

We hope you leave Trakošćan with new knowledge, new connections and ideas for what we can do together.

Ines Sviličić Petrić



President of the Organising Committee

Ana Bielen



President of the Scientific Committee

GENERAL INFORMATION

CONGRESS VENUE

The meeting is held at Hotel Trakošćan, Trakošćan 5, HR-42253 Bednja, Croatia.

REGISTRATION OF PARTICIPANTS

The registration desk will be open on Tuesday, 22 September from 12:00 to 14:00, and on every day of the congress from 08:30 to 09:00 in the lobby of Hotel Trakošćan. Daily updates on congress 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 congress social events.

LANGUAGE

The official language of the congress is English.

INTERNET ACCESS

To access the internet, please enquire at the reception desk of Hotel Trakošćan.

INFORMATION FOR PRESENTERS

All oral presentations will be held in the congress hall Julijana of Hotel Trakošćan. Please upload your presentations after registering or as early as possible before your scheduled presentation time. Technical staff will be available 15 minutes before the start of each session.

All posters will be displayed in the congress hall Julijana for three days, from Tuesday to Thursday, and should be mounted on Tuesday before 18:45. Poster presenters are kindly requested to be at their posters and available for discussion during both poster sessions.

PROGRAMME

TUESDAY, SEPTEMBER 22, 2026

	Arrival of participants and accommodation	
12:00 – 14:00	**Registration of participants** **Hotel lobby**	
12:00 – 14:30	Mounting posters on poster boards **Congress hall Julijana**	
14:30 – 15:00	**Opening ceremony** **Congress hall Julijana**	
15:00 – 16:00	**Opening Session** **Chaired by: Jasna Novak, Egon Bech Hansen** **Congress hall Julijana**	
15:00 – 16:00	KL1	**Giuseppe Spano** – Microbial innovation for a sustainable food future: *Lactiplantibacillus plantarum* as model
16:00 – 16:30	Coffee break (and mounting posters on poster boards)	
16:30 – 18:30	**Session 1: Microbes for the Healthy Planet** **Chaired by: Ines Sviličić Petrić, Damir Kapetanović** **Congress hall Julijana**	
16:30 – 17:00	IL1	**Denis Kutnjak** – Spatiotemporal Dynamics of Viromes in a River System Impacted by Wastewater Discharge
17:00 – 17:30	IL2	**Dubravko Pavoković** – First record and multilocus sequence typing of endosymbiontic bacteria *Wolbachia pipientis* in ants and spiders in Croatia
17:30 – 17:45	OP1	**Anđela Bačinić** – Assessment of the potential of bacterial isolates for the bioremediation of TBT-contaminated sediments
17:45 – 18:00	OP2	**Dunja Jurina** – Microbial diversity of river periphyton in the Pannonian ecoregion of Croatia
18:00 – 18:15	OP3	**Anastazija Hud** – A preliminary study of microbial dynamics in a common carp nursery pond

TUESDAY, SEPTEMBER 22, 2026

18:15 – 18:30	OP4	**Marko Marohnić** – Evaluation of viral enrichment strategies for compost Virome Analysis
18:30 – 18:45	SL1	**Tomasz Marasek** – From Colilert® to TECTA™: Advancing Microbiological Water Testing for Water and Wastewater Operations (IDEXX-sponsored lecture)
18:45 – 19:45	**Poster Session**	**Congress hall Julijana**
20:00	Welcome Reception	**Hotel Restaurant / Terrace**

WEDNESDAY, SEPTEMBER 23, 2026

08:30 – 10:30	**Session 2: Microbes for Human, Animal and Plant Health** **Chaired by: Maja Rupnik, Ana Bielen** **Congress hall Julijana**	
08:30 – 09:00	IL3	**Martin Hoenigl** – Impact of climate change on fungal diseases: from the pathogen to the host
09:00 – 09:30	IL4	**Martina Šeruga Musić** – Transcriptomic profiling of *Arabidopsis thaliana* transgenic lines overexpressing *'Candidatus Phytoplasma solani'* SAP-like effectors
09:30 – 09:45	OP5	**Ljudevit Luka Boštjančić** – Viromes of invasive crayfish (order Decapoda): insights from calico crayfish and signal crayfish across organs
09:45 – 10:00	OP6	**Marin Ježić** – Cryphonectria hypovirus 1 effect on its host physiology in various fungal isolates
10:00 – 10:15	OP7	**Marta Kozarić** – Qualitative proteomic analysis and antifungal susceptibility of clinical and environmental *Fusarium musae* isolates
10:15 – 10:30	OP8	**Ela Šarić** – Phenotypic and transcriptomic responses of *Aphanomyces astaci* to thermal stress
10:30 – 11:00	Coffee break	
11:00 – 12:30	**Meet the Editor Workshop** with **Iva Grabarić Andonovski** **Congress hall Julijana**	
12:30 – 14:00	Lunch break (optional) **Hotel restaurant**	
14:00 – 16:00	CDM1	AI-driven Microbiology with **Tomaž Curk**

WEDNESDAY, SEPTEMBER 23, 2026

16:00 – 16:30	Coffee break
16:30 – 17:30	**Poster Session** **Congress hall Julijana**
17:30 – 19:00	**Orange Data Mining Workshop** with **Tomaž Curk** **(limited to 20 participants with laptops)** **Congress hall Elizabeta**
20:00	Social programme: Pub Quiz / Paint & Wine / Štrukli baking **Congress hall Elizabeta / Hotel bar / Hotel restaurant**

THURSDAY, SEPTEMBER 24, 2026

08:30 – 10:30	**Session 3: Microbes and Biotechnology for Food Science and the Bioeconomy** **Chaired by: Andreja Leboš Pavunc, Giuseppe Spano** **Congress hall Julijana**	
08:30 – 09:00	IL5	**Egon Bech Hansen** – Cell envelope proteinases (CEP) of lactic acid bacteria
09:00 – 09:30	IL6	**Doris Marko** – The complex interaction between gut microbiota and mycotoxins
09:30 – 09:45	OP9	**Magdalena Hižak** – From indigenous bacteria to synthetic microbial consortia: a climate-smart strategy for traditional crop production
09:45 – 10:00	OP10	**Jana Fránová** – Viral and phytoplasma diseases of blueberries in the Czech Republic: from symptoms and vector occurrence to molecular detection and mapping of their spread
10:00 – 10:15	OP11	**Dijana Škorić** – An apple a day, and various plant pathogenic agents along the way
10:15 – 10:30	OP12	**Jasenka Petrić** – Microbiological safety of plant based dairy and meat substitutes at end of shelf life in Croatia
10:30 – 11:00	Coffee break	
11:00 – 12:00	KL2	**Ivan Mijaković** – Bacterial cell factories for biosustainability applications: from carbon capture to future foods **Chaired by: Gordana Maravić Vlahoviček, Giuseppe Spano**
12:00 – 13:00	Lunch break (optional) **Hotel restaurant**	
13:00 – 17:00	Excursion **Guided city tour of Varaždin**	
17:00 – 19:00	CDM2	Microbiology Meets Industry with **Iva Guberović** (Nuqleus), **Egon Bech Hansen, Dragomira Majhen** & **Martin Kavšček**
20:00	Gala Dinner **Hotel restaurant**	

FRIDAY, SEPTEMBER 25, 2026

09:30 – 12:00	**Session 4: Fundamental Microbiology for Future Applications** **Chaired by: Marina Svetec Miklenić, Davor Zahradka** **Congress hall Julijana**	
09:30 – 10:00	IL7	**Igor Stuparević** – Dynamic regulation shaping the *Saccharomyces cerevisiae* cell wall architecture and protein immobilization
10:00 – 10:30	IL8	**Ivana Ivančić-Baće** – Helicase activity and lysogeny influences the distribution of CRISPR-Cas spacer origins in *E. coli*
10:30 – 11:00	Coffee break	
11:00 – 11:15	OP14	**Jelena Repar** – Alternative end-joining mechanism contributes to the repair of DNA double-strand breaks in *Deinococcus radiodurans*
11:15 – 11:30	OP13	**Filip Božić** – Structural genome instability in *Deinococcus radiodurans* after extreme gamma-irradiation
11:30 – 11:45	OP15	**Dora Tomašković** – Artificial intelligence-enhanced FT-IR spectroscopy for rapid serovar identification of *Salmonella enterica subsp. enterica* isolates from Croatia
11:45 – 12:00	OP16	**Frederic Chaux** – Implementing a Synthetic Biology approach to unveil gene function across Marine Microalgae (SynBioMM): case-study of the vitamin B5 biosynthesis in coccolithophores
12:00 – 13:30	Lunch break (optional) **Hotel restaurant**	
13:30 – 15:30	CDM3	Microbiology Going Public with **Petra Buljević Zdjelarević** & **Marko Košiček** **Congress hall Julijana**

FRIDAY, SEPTEMBER 25, 2026

15:30 – 16:00	**Closing Ceremony – Awards for best poster and best short oral presentation** **Congress hall Julijana**
15:30 – 17:30	Microbes at Trakošćan – Public Microbiology Event for Children: Interactive hands-on stations introducing children to different microbiology topics **below hotel terrace**

KL	Keynote lecture
IL	Invited lecture
OP	Short oral presentation
SL	Sponsored lecture
CDM	Cross-disciplinary module

KEYNOTE LECTURES

KL1

Microbial Innovation for a Sustainable Food Future: *Lactiplantibacillus plantarum* as Model

Giuseppe Spano

DAFNE Department, University of Foggia, Italy

giuseppe.spano@unifg.it

The transition towards sustainable and resilient food systems demands innovative microbial solutions capable of simultaneously addressing food safety, nutritional quality, environmental sustainability, and human health. In this context, fermentation is undergoing a remarkable revitalisation—not only as an ancient preservation technology, but as a powerful bio-inspired platform for designing the next generation of foods, particularly within the rapidly growing plant-based sector.

Among lactic acid bacteria, *Lactiplantibacillus plantarum* stands out as a model of functional versatility. Its nomadic lifestyle, remarkable genomic plasticity, and widespread occurrence across plant ecosystems, fermented foods, and the human gastrointestinal tract have endowed this species with an exceptional capacity to adapt to diverse substrates and processing environments. Yet, this versatility is highly strain dependent, making the identification of robust and multifunctional strains a critical challenge for food innovation.

This presentation will explore how selected *L. plantarum* strains can drive sustainable food innovation through multiple complementary mechanisms, including stress tolerance, rapid acidification, bacteriocin production, bioconversion of plant substrates, synthesis of B-group vitamins and antioxidant metabolites, and degradation of antinutritional factors. Particular emphasis will be placed on the convergence of starter, protective, probiotic, and postbiotic functionalities, illustrating how microbial performance within food matrices may translate into beneficial effects on gut microbial ecology and host health.

By connecting microbial biodiversity with emerging food-system needs, *L. plantarum* provides a fascinating model for transforming fermentation into a strategic tool for resilience, circularity and health. The integration of multifunctional microbial cultures into food production has the potential to support safer and more nutritious plant-based foods, reduce resource inputs and food losses, and contribute to a sustainable, health-oriented bioeconomy. Ultimately, the future of fermentation may lie not only in preserving food, but in redesigning food systems themselves.

KL2

Bacterial Cell Factories for Biosustainability Applications: From Carbon Capture to Future Foods

Ivan Mijakovic

Chalmers University of Technology, Sweden
Technical University of Denmark, Denmark

ivan.mijakovic@chalmers.se

Rising CO_2 emissions are driving climate change, threatening both human and planetary health. For achieving the net zero goals set by most European countries, significant advances in carbon capture and storage technologies will be crucial, as well as developing new technologies that curb emissions related to agriculture. To contribute to these goals, the Mijakovic group has been working on a bio-based CO_2 capture technology that mimics the natural biomineralization process used by corals to form their exoskeleton. In this reaction dissolved CO_2 is first converted to carbonate (CO_3^{2-}), which reacts with calcium ions (Ca^{2+}) to form calcium carbonate ($CaCO_3$), a mineral that is practically insoluble in water. The conversion of CO_2 to carbonate is a bottleneck in the reaction, but it can be accelerated using the enzyme carbonic anhydrase (CA) as catalyst. Importantly, the overall reaction occurs optimally at alkaline pH 9. Anticipating that a large fraction of industrial CO_2 emissions will require a carbon capture process that is effective at high temperatures and high pH, we have engineered and patented a thermo- and alkali-tolerant strain of *Parageobacillus thermoglucosidasius* with surface display of CA, thereby accelerating the biomineralization process. This patented process is now undergoing scale-up with investment from Total Energies. In terms of replacing traditional agriculture, the Mijakovic lab has developed bacterial strains that produce collagens and caseins, for future food applications. The major challenge with producing these proteins in bacteria were their peculiar structures (triple helix for collagen, and disordered structure for casein), and their requirement for specific post-translational modifications (PTMs): hydroxylation of prolines for collagen, and serine/threonine phosphorylation for casein. Leveraging on our experience with PTMs in bacteria and synthetic biology approaches, we have achieved effective production strains that have been patented and are also undergoing scale up. While we have clearly demonstrated that microbe-based alternatives to traditional processes can be engineered, the next challenge will be establishing economic feasibility of these processes, and in some cases the major bottleneck will be consumer acceptance.

INVITED LECTURES

IL1

Spatiotemporal Dynamics of Viromes in a River System Impacted by Wastewater Discharge

Lana Vogrinec[1,2], Maja Ferle[1], Živa Lengar[1], Veronika Bukvič[1], Katarina Bačnik[1], Martina Bačič[3], Špela Erčulj[4], Carissa Bleker[1], Marjetka Levstek[4], Ion Gutiérrez-Aguirre[1], Carolyn M. Malmstrom[5], Denis Kutnjak[1]

[1]*National Institute of Biology, Ljubljana, Slovenia*
[2]*International Postgraduate School Jožef Stefan, Ljubljana, Slovenia*
[3]*Biotechnical Faculty, University of Ljubljana, Slovenia*
[4]*Wastewater Treatment Plant Domžale-Kamnik d.o.o., Domžale, Slovenia*
[5]*Michigan State University, East Lansing, MI, USA*

denis.kutnjak@nib.si

Rivers respond rapidly to environmental and anthropogenic pressures and integrate biological signals from the surrounding landscape. Their viromes remain underexplored, and it is still unclear how viral communities change over time, across connected environments, and in response to external stressors. We analyzed viromes across a connected system including a wastewater treatment plant (WWTP), representing a strong external stressor, the receiving river, and adjacent riparian vegetation over a two-year period. Monthly sampling captured wastewater and river water upstream and downstream of the WWTP, together with repeated sampling of wild plant communities along the same river stretch. Viral communities were characterized using high-throughput sequencing and analyzed in relation to spatial position, seasonality, and physicochemical parameters. Differences between wastewater, upstream, and downstream river viromes were consistent through time, with downstream samples showing clear similarity to wastewater, indicating a continuous influence of effluent discharge. Temporal patterns were structured by seasonality, with recurrent annual trajectories in community composition. At the same time, a subset of viruses was detected repeatedly across sampling points and conditions, indicating recurring presence across space and time. Strong short-term changes in physicochemical parameters were associated with distinct, but transient, shifts in virome composition. These shifts included changes in the relative abundance of plant-associated viruses. Multiple plant virus families were detected in wastewater and river water, and some of the same viruses were observed in plant samples within the same periods, indicating ongoing exchange between these compartments. These results show that river viromes reflect both strong continuous environmental stressors as well as short-term perturbations, while maintaining a reproducible structure over time. This provides a basis for using virome data to track ecosystem state and to study the movement of viruses beyond their primary hosts.

IL2

First Record and Multilocus Sequence Typing of Endosymbiontic Bacteria *Wolbachia pipientis* in Ants and Spiders in Croatia

Duje Lisičić[1], Mihaela Marceljak Ilić[2], Martina Šeruga Musić[1], Juraj Ulaga[1], Sabina Ranogajec Blažeka[1], Lana Marković[1], Marta Katinić[1], Julijana Matković[1], Marina Hajdarović[2], Dora Bedenic[2], Dubravko Pavoković[1]

[1]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*
[2]*XV. Gimnazija, Zagreb, Croatia*

dubravko.pavokovic@biol.pmf.unizg.hr

Wolbachia pipientis is the most widespread endosymbiotic bacterium in the animal kingdom. It exists as either a parasite or a mutualist in numerous arthropod species, as well as in dozens of nematode species. The predominant mode of transmission is vertical (maternal), though horizontal transmission is also possible. It has characteristic phenotypic effects on host reproduction and fertility that maximizes its spread. Ants are an important component in most terrestrial ecosystems. In this study, we investigated the occurrence and genetic diversity of *Wolbachia* isolates in ant populations from Croatia. A total of 97 ant samples were tested by polymerase chain reaction for the presence of the bacterial gene wsp. Positive samples were subjected to the multilocus sequence typing (MLST) analysis by amplification and sequencing of 5 house-keeping genes (*gatB*, *coxA*, *hcpA*, *fbpA* and *ftsZ*) in order to determine a specific sequence type (ST) according to the established MLST. Phylogenetic analysis of concatenated sequences was performed and *Wolbachia* was detected in 7 samples from 5 localities of two different Croatian regions: the continental region and the alpine region. Majority of the infected ant samples belonged to the genus *Lasius*. MLST analysis has revealed a great variability among 6 isolates that were successfully typed with isolates from different localities having a specific ST. Only one isolate corresponded to the already described ST, while 3 new STs having new allele genotypes for *coxA*, *ftsZ* and *gatB* genes were found. Moreover, phylogenetic analyses have shown the affiliation of typed isolates to the supergroup A. To the best of our knowledge, this is the first MLST analysis and the first record of Wolbachia isolates in ants in Croatia. Although the analyzed ants in Croatia were infected with *Wolbachia* with low frequency, our results have shown a considerable variability among identified isolates. In addition to ants, we continued our research on spiders, which play a role in the ecosystem where they contribute to biodiversity and ecosystem stability, and vertical and horizontal bacterial infection is possible in them. We sampled at four different locations in Slunj, Karlovac, Turopolje and Koprivnica. Out of 141 samples, 65 were processed, with *Wolbachia* found in 16 samples in genera: *Frontinellina* sp., *Mangora* sp., *Tibellus* sp., *Neottiura* sp. and *Oxyopes* sp. The goal is more knowledge about areas of ant and spider *Wolbachia* infestation in the Croatia.

IL3

Impact of Climate Change on Fungal Diseases: From the Pathogen to the Host

Martin Hoenigl

Division of Infectious Diseases, Department of Internal Medicine, Medical University of Graz, Graz, Austria

hoeniglmartin@gmail.com

The effects of climate change and natural disasters on fungal pathogens and the risks for fungal diseases remain incompletely understood. In this literature review, we examined how fungi are adapting to an increase in the Earth's temperature and are becoming more thermotolerant, which is enhancing fungal fitness and virulence. Climate change is creating conditions conducive to the emergence of new fungal pathogens and is priming fungi to adapt to previously inhospitable environments, such as polluted habitats and urban areas, leading to the geographical spread of some fungi to traditionally non-endemic areas. Climate change is also contributing to increases in the frequency and severity of natural disasters, which can trigger outbreaks of fungal diseases and increase the spread of fungal pathogens. The populations mostly affected are the socially vulnerable. More awareness, research, funding, and policies on the part of key stakeholders are needed to mitigate the effects of climate change and disaster-related fungal diseases.

IL4

Transcriptomic Profiling of *Arabidopsis thaliana* Transgenic Lines Overexpressing *'Candidatus Phytoplasma solani'* SAP-like Effectors

Dino Davosir[1], Iva Marković[1], Marina Drčelić[1], Rebeka Strah[2,3], Maruša Pompe Novak[2,4], Martina Šeruga Musić[1]

[1]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*
[2]*National Institute of Biology Ljubljana, Ljubljana, Slovenia*
[3]*Jožef Stefan International Postgraduate School, Ljubljana, Slovenia*
[4]*Faculty for Viticulture and Enology, University of Nova Gorica, Nova Gorica, Slovenia*

martina.seruga.music@biol.pmf.hr

Microbial effectors are small secreted molecules, predominately proteins, that alter host physiology, development and immune responses in order to facilitate and promote infection. Endocellular plant-pathogenic bacteria from the genus '*Candidatus Phytoplasma*' affect numerous plant species worldwide. Nevertheless, only a few phytoplasma effectors have been well characterized so far. In our previous research, we identified putative effector genes from genomes of *'Ca. P. solani'*, an important pathogen of grapevine and other economically relevant crops in the Euro-Mediterranean region. Furthermore, we generated transgenic arabidopsis (*Arabidopsis thaliana* (L.) Heynh) lines overexpressing effectors SAP11-like and SAP54-like that displayed significantly altered plant growth and development. The interaction of SAP11-like and SAP54-like effectors with plant transcription factors was also demonstrated. In the scope of this research, we aimed to examine the impact of SAP11-like and SAP54-like effectors on a transcriptomic level and to further identify their potential host targets. Total RNA was extracted from tissue of two-week-old seedlings of two independent lines for each effector as well as wild type, followed by quality assurance, library preparation with polyA enrichment and RNA sequencing. Bioinformatic analyses' results revealed considerable alterations in transcriptomes of all transgenic arabidopsis lines, compared to the wild-type. As shown by differential gene expression analyses, a substantial number of downregulated and upregulated genes was found, indicating disturbance of physiological and metabolic processes by SAP11-like and SAP54-like effectors. Photosynthesis-related genes together with genes involved in plant hormone transduction pathways and glucosinolate biosynthesis were the most downregulated ones, as revealed by functional KEGG enrichment analysis. Conversely, enhanced upregulation was found in phenylpropanoid biosynthesis genes, starch and sucrose metabolism and genes involved in plant-pathogen interaction pathways. Moreover, transcriptomic profiling revealed differences between SAP11-like and SAP54-like transgenic lines. Our results give the first insight into the general changes of plant

transcriptome in response to *'Ca. P. solani'* effectors. Further studies will aim to reveal new potential effector targets and phytoplasma pathogenicity mechanisms and offer a foundation for novel disease management strategies.

IL5

Cell Envelope Proteinases (CEP) of Lactic Acid Bacteria

Egon Bech Hansen

National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark

egbh@food.dtu.dk

The bacteria commonly known as the lactic acid bacteria (LAB) constitute the order Lactobacillales. Several of the genera and species in this order are utilized for the production of fermented foods. Several are listed as safe on the QPS list of EFSA and the GRAS inventory of FDA. However, it should be noted that Lactobacillales also harbor deadly pathogens.

Cell envelope proteases (CEP) are widespread but not ubiquitous among the Lactobacillales. The CEP enzymes, if present, typically gives the bacterium a weak proteolytic phenotype allowing the bacterium to selectively hydrolyze some proteins of the environment.

The CEP enzymes of Lactobacillales are large multidomain enzymes secreted to the outside of the cell and attached to the surface of the bacterium. The size of CEP enzymes ranges from around 1100 amino acids to lengths exceeding 2000 aa.

The proteolytic domain of the CEP enzymes has the same structural fold as the small unspecific serine protease, subtilisin. In addition to the proteolytic domain, CEP enzymes have domains limiting proteolytic activity and providing specificity. Swapping the A-domains between different *Lactococcus* CEP enzymes was found to give altered substrate specificity.

CEP enzymes differ in substrate specificity ranging from enzymes specific for a single site in a single peptide to enzymes hydrolyzing several proteins at multiple sites.

Pathogenic streptococci have evolved CEP enzymes into virulence factors. CEP virulence factors cause the immune system of the host to be jammed by selectively inactivating specific complement factors or cytokines.

The similarity in architecture and structure of the CEP enzymes was used as a basis for a strategy to identify inhibitors of these virulence factors. A high throughput screening using milk acidifying LAB strains allowed the identification of compounds inhibiting the CEP enzymes PrtP from *Lactococcus* and PrtS from *Streptococcus thermophilus*. Subsequently those inhibitors was tested for inhibition of ScpA of *Streptococcus pyogenes*.

Our current research on CEP enzymes for food cultures pursues the target of identifying LAB strains able to use plant proteins for supporting growth of the culture and generate a product with desirable taste and texture. Simultaneously we also pursue research on CEP virulence factors targeting the development of novel methods to control streptococcal infections in livestock and humans.

IL6

The Complex Interaction Between Gut Microbiota and Mycotoxins

Doris Marko, Francesco Crudo

Department of Food Chemistry and Toxicology, University of Vienna, Austria

doris.marko@univie.ac.at

Mycotoxins represent the most frequently occurring natural food contaminants. Mycotoxins are secondary metabolites of fungi, often formed to achieve an advantage in the respective biotope. Consequently, it is reasonable to conclude that humans don´t represent the original "targets" but that at least some of these secondary metabolites are formed to suppress competing microbial organisms. But if these compounds are present in the human diet on a regular basis, this might be of consequence for the composition of the gut microbiome. On the other hand, microbial metabolic activity in the gut might affect ingested mycotoxins with consequences for the intestinal uptake of the toxin and/or the bioactivity of the compounds. Recent studies demonstrate the existence of such a bidirectional relationship between mycotoxins and the gut microbiome. Emerging mycotoxins formed by *Alternaria* spp. are frequently detected in fruits, cereals, infant foods, and human breast milk. Risk assessment of *Alternaria* toxins is challenging due to the complexity of secondary metabolites formed by these fungi. Depending on the *Alternaria* strain, substrate and environmental conditions, the toxin composition may vary substantially and only a limited number of toxins are commercially available for hazard characterization. Complex extracts of *Alternaria alternata* have been shown to possess genotoxic and immunosuppressive properties in vitro. But the DNA strand breaking properties of such extracts were found to be almost completely quenched by short time incubation with fecal slurry. The suppression of the genotoxic properties was found to be a direct consequence of the reduction of mycotoxin concentrations in samples analyzed by LC–MS/MS. Further investigations revealed the ability of human gut bacterial strains (especially the gram-negative ones) to adsorb the most lipophilic *Alternaria* mycotoxins thus potentially reducing the free absorbable proportion of mycotoxins. On the other hand, complex *Alternaria* mycotoxin extracts were found to affect the growth of several bacterial strains and their ability to produce biofilms. So far, several *Alternaria* toxins with antimicrobial activity have been identified. Taken together, these findings highlight the potential role of mycotoxins in impacting the gut microbial community and, vice versa, the importance of the latter in modifying the systemic bioavailability of this class of food contaminants.

IL7

Dynamic Regulation Shaping the *Saccharomyces cerevisiae* Cell Wall Architecture and Protein Immobilization

Romana Ivković, Antonia Paić, Tea Martinić Cezar, Bojan Žunar, Renata Teparić, Igor Stuparević

Laboratory for Biochemistry, University of Zagreb Faculty of Food Technology and Biotechnology, Zagreb, Croatia

istuparevic@pbf.hr

The *Saccharomyces cerevisiae* cell wall is a stratified extracellular matrix that provides mechanical protection and mediates environmental sensing. Its architecture comprises an inner β-1,3-glucan–chitin network overlaid with mannoproteins, connected through diverse covalent and non-covalent interactions. Our work maps the molecular mechanisms shaping this structure, revealing how proteolytic maturation and ncRNA regulation coordinate cell wall remodelling and stability.

We identify a multi-step pathway controlling the glycoside hydrolase Scw4: sequential cleavage by the Golgi protease Kex2 and cell-surface yapsins activates the enzyme while reducing its covalent attachment to glucan, enabling dynamic restructuring. Cell wall integrity also depends on the nuclear RNA exosome (Rrp6 and Dis3). Loss of exosome function causes osmoremedial lysis at elevated temperatures due to defective protein glycosylation. This defect arises from reduced expression of the essential mannosylation enzyme Psa1, triggered by the accumulation of sense ncRNAs at its promoter.

Long non-coding RNAs further refine the expression of nearly half of all cell wall-related genes through transcriptional interference and chromatin modulation. At the *PHO5* locus, antisense transcription recruits the Rpd3 deacetylase, generating a repressive chromatin state that limits RSC access and delays induction.

These insights into native immobilisation and regulatory pathways inform Yeast Surface Display engineering. Using anchors such as Pir proteins or the GPI-linked Ccw12, we developed quantitative β-lactamase reporters to measure display capacity and catalytic activity *in situ*. Building on this platform, we are engineering the probiotic *Saccharomyces boulardii* to display sweet proteins directly on the cell surface, eliminating purification steps and enabling incorporation into functional foods that combine high-intensity sweetness with probiotic benefits.

IL8

Helicase Activity and Lysogeny Influences the Distribution of CRISPR-Cas Spacer Origins in *E. coli*

Mirta Žagar, Tomislav Mamić, Damjan Mitić, Marta Novaković, Ivana Ivančić-Baće

Department of Biology, Faculty of Science, University of Zagreb, Croatia

ivana.ivancic.bace@biol.pmf.hr

The CRISPR-Cas adaptive immune system protects many bacteria and most archaea from invading mobile genetic elements (MGEs) by acquiring, storing, and exploiting molecular memories of previous encounters for defence. During naïve adaptation, short DNA fragments derived from MGEs, termed spacers, are integrated into CRISPR arrays, which consist of alternating repeat and spacer sequences. This process depends on the highly conserved Cas1-Cas2 heterohexameric integration complex and the coordinated action of host DNA repair and replication machinery. Our recent study has shown that replication and post-replicative DNA gaps generate DNA substrates that stimulate better binding of Cas1-Cas2. On the other hand, replication fork stalling and collapse stimulate DNA repair by the RecBCD recombination pathway that also produce DNA substrates that are captured, annealed, trimmed, and integrated into the CRISPR locus by Cas1-Cas2.

Spacer origins are not randomly distributed across the genome but are enriched at three chromosomal hotspots, most prominently around the origin of replication (*oriC*), the CRISPR array, and the replication terminus (*Ter*) region. In this study we performed a high-resolution bioinformatic analysis of newly acquired spacers from two helicase mutants (*recG* and *recQ*), DNA repair mutant (*recF*), DNA repair and helicase double mutants (*recQ recF*, *recF recG*) and two λ lysogens (λ^+ and λ^c). We present new data demonstrating how these mutations affect spacer origin preferences (plasmid, prophage, or chromosomal DNA), the genomic distribution of spacer acquisition events, relative spacer acquisition efficiencies, and spacer lengths. The most pronounced effects were observed in the *recF* and *recG* mutants, providing new insights into the molecular mechanisms underlying CRISPR-Cas adaptation.

SHORT ORAL PRESENTATIONS

OP1

Assessment of the Potential of Bacterial Isolates for the Bioremediation of TBT-contaminated Sediments

Anđela Bačinić[1], Goran Palijan[2], Ivna Vrana[1], Ines Sviličić Petrić[1], Anastazija Huđ[1], Martina Furdek Turk[1]

[1]*Ruđer Bošković Institute, Department for Marine and Environmental Research Zagreb, Croatia*
[2]*Josip Juraj Strossmayer University of Osijek, Department of Biology Osijek, Croatia*

mfurdek@irb.hr

Tributyltin (TBT) is a toxic biocidal organotin compound used for decades in antifouling paints. Due to its high toxicity, the EU Water Framework Directive classifies it as a priority hazardous substance. Although prohibited, TBT is still present in many coastal areas, especially in marine sediment, where it degrades slowly. The degradation of TBT varies between sediment types, but the reasons for these differences are not fully understood, and the respective roles of abiotic processes and microbial communities in TBT degradation remain unclear. Within the project "Transformations, mobility, and persistence of organotin compounds in the marine environment", the influence of microbial communities on TBT degradation is investigated. The specific aim of this study is to isolate TBT-resistant bacteria from sediments and to investigate their potential for degradation of TBT in contaminated sediments.

For the purpose of this study, TBT-tolerant bacteria were isolated from eight marine sediment samples collected along the Adriatic coast at sites exhibiting different levels of contamination. Isolation was performed on marine agar plates supplemented with TBT (50 μg L^{-1}). Pure cultures were obtained, and total DNA was extracted and subjected to Sanger sequencing for taxonomic identification. In total, 28 bacterial isolates were recovered from the sediment samples, and classified into six taxonomic groups. While the genera *Pseudoalteromonas*, *Pseudomonas*, and *Bacillus* are already reported in the literature as potential TBT-degrading bacteria, the isolates *Thalassospira* sp., *Corallincola spongiicola*, *Marinobacterium rhizophilum*, and *Oceanobacillus massiliensis* obtained in this study have not been previously associated with TBT degradation processes in sediments.

The potential of bacterial isolates for TBT degradation were tested in two types of liquid media, seawater-based minimal medium with and without glucose supplementation. Changes in TBT concentrations and the formation of its degradation products will be used to determine degradation kinetic constants and to assess the degradation potential of the investigated isolates. In the next phase of the study, their ability to degrade TBT in marine sediments will be

assessed in order to determine their actual potential for the bioremediation of contaminated sediments.

This work was supported by the Croatian Science Foundation under the project number HRZZ-IP-2024-05-1906.

OP2

Microbial Diversity of River Periphyton in the Pannonian Ecoregion of Croatia

Dunja Jurina[1], Antonija Kulaš[1], Petar Žutinić[1], Csilla Stenger-Kovács[2], Ines Sviličić Petrić[3], Petra Pjevac[4], Marija Gligora Udovič[1]

[1]*Faculty of Science, University of Zagreb, Croatia;*
[2]*University of Pannonia, Limnology Research Group*
[3]*Ruđer Bošković Institute, Zagreb, Croatia*
[4]*University of Vienna, Vienna, Austria*

dunja.jurina@biol.pmf.hr

River ecosystems play a crucial role in supporting ecological processes and maintaining biodiversity, but are increasingly exposed to anthropogenic pressures. Microbial communities are essential components of aquatic biogeochemical processes and are fundamental to ecosystem functioning; among them, periphytic communities are particularly valuable as sensitive indicators of environmental conditions and ecosystem health. However, research on riverine microbial diversity has predominantly focused on seasonal changes in planktonic communities, while periphytic communities have received less attention. In this study, we characterized periphyton diversity across 50 sites in 12 rivers of Pannonian ecoregion in Croatia, encompassing three major regions: Danube River, Sava region (Sava, Kupa, Una, Odra, Orljava, and Krapina rivers) and Drava region (Drava, Bednja, Mura, Plitvica, and Karašica rivers).Community composition and diversity were determined by amplifying the hypervariable V4-region of the 16S SSU rRNA gene for prokaryotes and the hypervariable V9-region of the 18S SSU rRNA gene for eukaryotes. The prevalent bacterial classes were Cyanobacteria, Alphaproteobacteria, Gammaproteobacteria, Verrucomicrobia, Bacteroidia, and Planctomycetes. Cyanobacteria emerged as the dominant class during both seasons in 2022, whereas their relative abundance decreased in 2024. The dominant eukaryotic classes included Bacillariophyta, Ulvophyceae, Chlorophyceae, Phyllopharyngea, and Oligohymenophorea. Bacillariophyta was predominant, although some samples had low abundance (<5.00%). Non-metric multidimensional scaling (NMDS) demonstrated that in both Sava and Drava region, centroids corresponding to different years were distinct. Additional differentiation occured between seasons, suggesting consistent temporal shifts in prokaryotic and eukaryotic community compositions. Furthermore, NMDS analysis conducted on complete dataset revealed a distinct separation of the Danube River community from the Sava and Drava regions. These findings offer new insights into freshwater periphyton communities in the Pannonian ecoregion and enhance understanding of seasonal dynamics and diversity in large Croatian

rivers. Such knowledge of freshwater microbial diversity is crucial for effective biodiversity conservation and sustainable resource management, especially given the rapid decline of freshwater biodiversity.

OP3

A Preliminary Study of Microbial Dynamics in a Common Carp Nursery Pond

Frederic Chaux[1], Ivona Mladineo[2, 3], Anastazija Huđ[1], Ines Sviličić Petrić[2], Ivan Fiala[2], Ivana Babić[1]

[1]*Ruđer Bošković Institute, Zagreb, Croatia*
[2]*Institute of Parasitology, Biology Centre of the Czech Academy of Sciences (BC CAS), Budějovice, Czech Republic*
[3]*Institute for Marine and Antarctic Studies, University of Tasmania, Taroona, Tasmania, Australia*

ibabic@irb.hr

Common carp (*Cyprinus carpio*) pond farming represents a long-established aquaculture practice in Central and Eastern Europe, combining fish production with valuable ecosystem functions. Despite the recognized ecological and socioeconomic value of pond ecosystems, there is still limited knowledge regarding how environmental fluctuations shape microbial communities and affect fish health. In this study, we investigated microbial succession and resilience within a common carp nursery pond using full-length 16S rRNA Nanopore sequencing. Microbial dynamics were monitored across manure, sediment, water, and fish-associated habitats (gut and gills).

Our results revealed that chicken manure acted as an important source of microbial inoculation, introducing several allochthonous bacterial genera like *Acinetobacter* and *Geofilum* into the pond ecosystem. While the water column maintained a relatively stable microbial structure dominated by *Limnohabitans*, sediment and fish-associated microbiomes underwent pronounced restructuring in response to environmental stress. A summer heatwave (30 °C) triggered a 53% decline in sediment richness and coincided with the lowest mucosal diversity observed in fish, alongside an enrichment of opportunistic *Pantoea*. Despite this disruption, microbial communities showed a strong capacity for recovery, with diversity restored by the drawdown period and communities reassembled around environment-derived taxa such as *Polynucleobacter* and *Lysinibacillus*.

These findings demonstrate the strong influence of management practices and climatic events on pond microbiomes while highlighting the capacity of the pond holobiome to recover after acute environmental stress. Improved understanding of these microbial processes may contribute to the development of more resilient and sustainable aquaculture systems under future climate-change scenarios.

OP4

Evaluation of Viral Enrichment Strategies for Compost Virome Analysis

Marko Marohnić[1,2], Ion Gutiérrez-Aguirre[1], Ana Vučurović[1], Maja Ferle[1], Lana Vogrinec[1,2], Denis Kutnjak[1]

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

marko.marohnic@nib.si

Compost is a nutrient-rich organic fertilizer that forms through the aerobic decomposition of organic materials such as food waste, plant residues, and household waste. Although compost supports crop production and environmental sustainability, it can also pose phytosanitary risks because it may contain environmentally stable plant RNA viruses that persist outside their hosts and remain infectious. Detecting these viruses is challenging because abundant background nucleic acids obscure viral sequences in high-throughput sequencing (HTS) datasets, and organic inhibitors interfere with reverse transcription quantitative PCR (RT-qPCR) and HTS library preparation. Therefore, efficient viral enrichment becomes necessary before downstream analyses. To identify an efficient viral enrichment approach for compost samples, we tested and compared ultracentrifugation, ultrafiltration, flocculation with polyethylene glycol (PEG) and chromatography-based concentration methods, combined with application of nuclease enzymes. We designed a modular protocol that contains several homogenization and filtration steps of compost samples. To compare the methods, we used specific RT-qPCR assays for detection of viruses spiked at the beginning of the protocol as well as fungal and bacterial background nucleic acids. Additionally, we utilized HTS on Illumina platform for samples concentrated with PEG flocculation and analysed resulting data utilizing different bioinformatic pipelines to study the effects of viral spiking and nuclease treatment. RT-qPCR results showed that nuclease treatment reduced bacterial and fungal nucleic acids while maintaining recovery of the spiked viruses. However, all concentration methods showed strong PCR inhibition, which prevented reliable quantitative comparison. Recovery of spiked viruses varied among methods, and no single method consistently outperformed the others. HTS analysis of PEG-concentrated samples showed that nuclease treatment increased viral reads from 6.33% to 11.26%, mainly driven by DNA viruses, but reduced recovery of both spiked and naturally occurring RNA viruses. These results show that nuclease treatment improves viral enrichment by reducing background nucleic acids, although further optimization is needed to improve

RNA virus recovery. Strong PCR inhibition highlights the need for improved inhibitor removal strategies to establish a robust HTS-based workflow for plant virus detection and characterization in compost.

OP5

Viromes of Invasive Crayfish (Order Decapoda): Insights from Calico Crayfish and Signal Crayfish Across Organs

Ljudevit Luka Boštjančić[1,2], Andrea Belamarić[2], Katarina Bačnik[2], Ana Bielen[4], Antonin Kouba[3], Sandra Hudina[5], Denis Kutnjak[2]

[1]*Ecology Department, Faculty of Biology, RPTU Kaiserslautern-Landau, Kaiserslautern, Germany*
[2]*National Institute of Biology (NIB), Ljubljana, Slovenia*
[3]*Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in České Budějovice, Vodňany, Czech Republic*
[4]*Department of Biochemical Engineering, University of Zagreb Faculty of Food Technology and Biotechnology, Zagreb, Croatia*
[5]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*

Luka.Bostjancic@nib.si

Invasive freshwater crayfish are major drivers of biodiversity loss in European freshwaters, yet the role of their associated viral communities (viromes) in invasion success remains poorly understood. Viruses may influence invasion dynamics through reduced pathogen pressure in the new range (enemy release), pathogen spillover to native species, or direct effects on host fitness. Despite growing interest, crayfish viromes are largely uncharacterised, and the optimal biological matrices for their detection and monitoring have not been established. Here we present virome data from two prominent North American invaders in Europe: the calico crayfish (*Faxonius immunis*) and the signal crayfish (*Pacifastacus leniusculus*). For calico crayfish, eight individuals collected at Lauterbourg (France, Rhine River system) were analysed across multiple organs (gills, gut, and hepatopancreas), alongside environmental samples and cuticular swabs. For signal crayfish, multi-organ samples (gills, hepatopancreas, green gland, muscle) were collected at Korana River (Croatia). Sequencing libraries were generated following rRNA depletion and subjected to shotgun RNA sequencing on the Illumina platform. Bioinformatic analyses included *de novo* assembly, similarity searches against the NCBI sequence databases, taxonomic classification and phylogenetic inference.

Preliminary analyses of calico crayfish revealed multiple putative RNA viral contigs, showing 29.96–66.16% nucleotide identity to reference viruses in the NCBI RefSeq Viral database, suggesting the presence of potentially novel viral taxa. In contrast, sequencing datasets from signal crayfish were dominated by previously described viruses associated with this host, including signal crayfish-associated reo-like virus 1, toti-like virus 1, and hepe-like virus 1. Organ-specific

patterns were observed in both species and will be integrated into a comparative framework to identify optimal sampling matrices and assess shared viral diversity across species.

These findings lay the groundwork for cross-species comparisons of virome composition across organs in invasive crayfish. The data will inform optimal sampling strategies for crayfish virome surveillance and contribute to understanding the role of viruses in freshwater invasion dynamics and cross-species pathogen transmission.

OP6

Cryphonectria hypovirus 1 Effect on Its Host Physiology in Various Fungal Isolates

Marin Ježić[1], Lucija Nuskern[1], Karla Peranić[1], Maja Popović[2], Rosemary Vuković[3], Zorana Katanić[3], Ljiljana Krstin[3], Deborah Marie Leigh[4], Igor Poljak[2], Marilena Idžojtić[2], Daniel Rigling[4], Mirna Ćurković-Perica[1]

[1]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*
[2]*Faculty of Forestry and Wood Technology, University of Zagreb, Zagreb, Croatia*
[3]*Department of Biology, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia*
[4]*Swiss Federal Research Institute WSL, Birmensdorf, Switzerland*

marin.jezic@biol.pmf.hr

Cryphonectria parasitica-Cryphonectria hypovirus 1 (CHV1) pathosystem has been a well-established and thoroughly studied model biocontrol system over the last several decades. The mycovirus suppresses the fungal pathogen's virulence, thus affecting its impact on the sweet chestnut host, a phenomenon known as hypovirulence. The study of this system was historically limited to only a few prototypical CHV1-infected fungal strains: Euro7 infected with the Italian (I) and EP713 infected with the French 1 (F1) subtype of the virus, collected a decades ago and maintained in laboratories across the world for years. In our studies, beyond these well-known fungal strains, we have analysed the impact of CHV1 infection on several recently collected "wild" fungal strains. For this purpose, oxidative stress parameters were used as a proxy to assess the biotic stress exerted by CHV1 on its host. Our analyses have indicated significantly different fungal responses to the biotic stress of viral infection between these prototypical and "wild" fungal strains, indicating reduced capacity of the prototypical strains to resist the aforementioned stress. Interestingly, the intra-host viral population diversity did not correlate with most of the measured stress level parameters. The wild analysed CHV1-infected fungal isolates had significant variability in oxidative stress level parameters, but no clear trend was apparent among them. Finally, we studied the effect of vertical virus transmission (i.e., into spores) and found that the changes in the stress level parameters between the parental mycelium and the mycelium derived from the CHV1-infected spores changed only intermittently. These findings indicate that the prototypical CHV1-infected fungal isolates seem to have a diminished capacity to defend against the biotic stress caused by the viral infection when compared to the "wild" fungal strains and that this innate characteristic is usually maintained during the vertical virus transmission.

OP7

Qualitative Proteomic Analysis and Antifungal Susceptibility of Clinical and Environmental *Fusarium musae* Isolates

Marta Kozarić[1], Ida Ćurtović Pučar[1], Daniela Jakšić[1], Karla Košpić[2], Željka Stanečić[2], Sanja Pleško[3], Marko Siroglavić[3], Zrinka Bošnjak[3], Cornelia Lass-Flörl[4], Martin Hoenigl[5], Maja Šegvić Klarić[1]

[1]*Department of Microbiology, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia*
[2]*Central Laboratory, BICRO BIOCENTRE Ltd., Zagreb, Croatia*
[3]*University Hospital Zagreb, Zagreb, Croatia*
[4]*Institute of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria*
[5]*Division of Infectious Diseases, Department of Internal Medicine, Medical University of Graz, Graz, Austria*

maja.segvic@pharma.unizg.hr

Fusarium musae is an emerging member of the *F. fujikuroi* species complex associated predominantly with banana-related environments and increasingly with human infections. Due to its potential environmental adaptation and opportunistic pathogenicity, this study investigated antifungal susceptibility patterns and qualitative proteomic profiles of clinical and environmental *F. musae* isolates.

A total of 30 *F. musae* isolates were analysed, including clinical strains (*n*=6) from Croatia and Austria and isolates (*n*=24) originating from banana. Species identification was performed using *TEF1-α*/*RPB2* sequencing and MALDI-TOF MS. Antifungal susceptibility testing according to CLSI guidelines included amphotericin B (AMB), voriconazole (VOR), posaconazole (POS), isavuconazole (ISA), and two novel animycotics olorofim (OLF) and manogepix (MGX). For proteomic analysis, one clinical isolate and one isolate from banana were cultured under *in vitro* / *ex vivo* conditions. Qualitative proteomic profiling was performed using nanoLC system coupled to a TripleTOF® 6600+ Sciex mass spectrometer.

VOR showed the lowest MICs among azoles, with values of 2–4 mg/L in most isolates. POS MICs ranged from 1–16 mg/L in clinical isolates and 0.25–2 mg/L in most banana isolates, whereas ISA showed consistently elevated MICs. AMB MICs ranged from 2 to >16 mg/L in clinical isolates. OLF demonstrated strong activity (0.125–2 mg/L), while MGX showed very low MIC_{50}/MEC values overall (<0.03 mg/L). Comparative analysis identified more than 400 proteins shared between clinical and banana isolates, including proteins involved in oxidative stress response (HSP10, HSP60, HSP70), cell wall-related processes (disulfide isomerases, ATPase-related transporters), as well as secondary metabolism

(FUB cluster and CFEM-related proteins). Notably, an RNB domain-containing protein was detected exclusively in the clinical isolate under *ex vivo* conditions. The efficacy of the novel antifungals OLF and MGX appears promising for the treatment of invasive mycoses caused by *F. musae*. Several proteins were shared between clinical and environmental isolates, while the exclusive detection of an RNB domain-containing protein in the clinical isolate under *ex vivo* conditions suggest potential adaptive mechanisms associated with clinical survival.

This work has been supported by the Croatian Science Foundation (HRZZ-IP-2022-10-8062; DOK-NPOO-2023-10-2348).

OP8

Phenotypic and Transcriptomic Responses of *Aphanomyces astaci* to Thermal Stress

Ela Šarić[1], Simone Oberhaensli[2], Heike Schmidt-Posthaus[2], Ana Bielen[1]

[1]*University of Zagreb Faculty of Food Technology and Biotechnology, Zagreb, Croatia*
[2]*Institute for Fish and Wildlife Health, University of Bern, Vetsuisse Faculty, Bern, Switzerland*

abielen@pbf.hr

Disease occurrence results from dynamic interactions among the host, pathogen, and environment, with temperature being a key factor influencing host–pathogen interactions. *Aphanomyces astaci*, the aquatic oomycete causing crayfish plague, is responsible for severe declines of native crayfish populations, yet the effects of temperature on pathogen development and transmission remain poorly understood. Its life cycle includes zoospores, cysts, and mycelium, responsible for dispersal, infection, and disease development. Five *A. astaci* genotypes (A–E) exist, differing in host, virulence, and geographic distribution.

We have investigated the effects of different temperature regimes on mycelial growth of *A. astaci* genotypes A–E, including constant temperatures (18–30 °C), daily fluctuations (22 ± 3 °C), and a heat wave (22 → 28 → 22 °C). Zoospore motility and cyst germination of genotypes A and D were evaluated at 18, 22 and 26 °C. Temperature-, genotype- and life stage-dependent transcriptional responses were assessed using RNA sequencing.

Genotypes B, C and D maintained stable mycelial growth between 18 and 26 °C, with growth declining only at 28 °C. In contrast, genotypes A and E were more sensitive to elevated temperatures, exhibiting reduced or completely inhibited growth under increasing constant temperatures as well as under fluctuating and heat-wave conditions. Consistent with these patterns, zoospore motility persisted significantly longer at 26 °C in genotype D than in genotype A. However, cyst germination rates remained similar across all tested temperatures in both genotypes, suggesting that infection potential may persist even when zoospore and mycelial viability are reduced. Further, RNA-seq analyses revealed that developmental stage was the primary driver of transcriptomic variation in *A. astaci*. Nevertheless, genotype and temperature regime also shaped the transcriptional response. In particular, genotype A showed a stronger temperature-dependent transcriptional response to genotype D, consistent with its lower thermal tolerance at the phenotypic level.

In conclusion, this study improves our understanding of how temperature influences the biology of *A. astaci* across developmental stages and genotypes. By combining phenotypic and transcriptomic approaches, the results provide

valuable insights into the mechanisms underlying thermal responses of this pathogen and contribute to predicting crayfish plague risks under future climate change scenarios.

This work has been supported by Croatian Science Foundation (IPCH-2024-04-6513) and Swiss National Science Foundation (310030L_219521).

OP9

From Indigenous Bacteria to Synthetic Microbial Consortia: A Climate-Smart Strategy for Traditional Crop Production

Magdalena Hižak[1], Dubravka Havaš[2], Jelena Šarac[2], Mario Lovrić[2], Maja Ivanić[1], Sanja Frka Milosavljević[1], Dunja Šamec[3], Ivana Rajnović[4], Ivan Magdić[4], Marko Petek[4], Goran Palijan[5], Ivana Babić[1], Ines Sviličić Petrić[1]

[1]*Ruđer Bošković Institute, Zagreb, Croatia*
[2]*Institute for Anthropological Research, Zagreb, Croatia*
[3]*University North, Croatia*
[4]*Faculty of Agriculture, University of Zagreb, Zagreb, Croatia*
[5]*Josip Juraj Strossmayer University of Osijek, Osijek, Croatia*

magdalena.hizak@irb.hr

The agricultural sector faces the challenge of feeding a growing population while adapting to increasing climate pressures. Drought remains one of the major causes of crop yield losses, threatening the sustainability of traditional agricultural systems. The CLIMAGRIT project addresses this challenge by integrating the selection of bioinocula from indigenous microorganisms, laboratory and field validation, and climate-risk modelling to improve the resilience of traditional crops from Varaždin County, Croatia, including Varaždin cabbage, Ludbreg horseradish and local pumpkin varieties.

Plant growth-promoting bacteria (PGPB) offer a promising strategy for improving crop performance under climate stress. A total of 185 bacterial isolates were obtained from a five-week greenhouse experiment using cabbage (*Brassica oleracea* L. var. *capitata* f. *alba*) as a model plant. Isolates were identified and screened for plant growth-promoting traits, including biocontrol activity, tolerance to abiotic stress, biofilm formation and exopolysaccharide production, and nutrient mobilisation through phosphorus and potassium solubilisation, ammonia production and organic acid synthesis.

Based on these characteristics, 62 isolates belonging to the genera *Bacillus*, *Enterobacter*, *Paenarthrobacter*, *Pantoea*, *Pseudomonas* and *Stenotrophomonas* were selected for further evaluation. Their drought tolerance was assessed under four polyethylene glycol (PEG) concentrations (0, 5, 10 and 15%). Artificial intelligence-assisted approaches were then used to construct synthetic microbial consortia (SynComs) based on the diversity of PGP traits and drought tolerance. Compatibility among consortium members was evaluated using cross-streak assays.

In the next phase, SynCom performance will be tested under drought and heat stress in growth-chamber and field experiments. Plant performance, soil properties, microbiome dynamics and crop quality will be monitored, while

machine-learning models integrating microbiome, sensor and climate data will be developed for early drought-risk prediction. Through this integrated approach, CLIMAGRIT aims to enhance the resilience of traditional crops to climate stress and support the long-term sustainability of regional agricultural production.

OP10

Viral and Phytoplasma Diseases of Blueberries in the Czech Republic: From Symptoms and Vector Occurrence to Molecular Detection and Mapping of Their Spread

Jana Fránová, Jaroslava Přibylová, Jana Veselá, Josef Špak, Igor Koloniuk

Institute of Plant Molecular Biology, Biology Centre, Czech Academy of Sciences, Czech Republic

jana@umbr.cas.cz

Blueberries have the highest antioxidant content of any fruit or vegetable. They are a rich source of vitamins A, C, and K, as well as minerals (Mn, K, Fe). They significantly boost the immune system and support brain function, and they also protect the eyes and blood vessels. At the same time, they have a low glycemic index and help maintain healthy blood sugar levels.

In 2024, we launched a project focusing on the prevalence of viruses and phytoplasmas in plants of the genus *Vaccinium*, including the development of diagnostic methods. Plants showing suspected viral and phytoplasma resembling symptoms as well as asymptomatic ones were sampled. To date, samples have been collected from more than 160 *V. corymbosum* plants from production fields, garden retailers and private gardens and more than 110 *V. myrtillus* plants growing in forests. The plants were tested for the presence of viruses and phytoplasmas using molecular methods. Sequences were compared with data available in GenBank to confirm the identity of the pathogens and to gain insights into their genetic variability.

Although blueberry bush dieback and significant economic damage caused primarily by blueberry leaf mottle, blueberry necrotic shock, blueberry scorch and blueberry shoestring viruses have been reported in the *V. corymbosum* native habitat in North America, these viruses have not yet been detected in the Czech Republic.

Data on discovery of the most frequently found viruses (blueberry virus L and blueberry latent virus) will be presented as well as on three new viruses identified using HTS. Two are widespread in *V. myrtillus*, and one was identified in a *V. corymbosum* seedling. In some commercial blueberry stands in Bohemia, as well as in purchased seedlings, the potential virus vector, aphid *Ericaphis scammelli* was identified using molecular and morphological methods. A survey focused on the occurrence of phytoplasmas revealed the presence of a *Vaccinium* sp. witches' broom phytoplasma (16SrIII-F), ´*Candidatus Phytoplasma asteris*´ (16SrI-B) and ´*Ca. P. trifolii*´ (16SrVI-A) in the wild growing blueberry bushes with proliferation symptoms.

The research was supported by Ministry of Agriculture of the Czech Republic, project no. QL24020033 and by institutional support from RVO60077344. The authors would like to express their sincere thanks to Dr. Jan Havelka for his valuable advice and for the morphological identification of the aphids.

OP11

An Apple a Day, and Various Plant Pathogenic Agents Along the Way

Dijana Škorić[1], Paolo Margaria[2], Ivana Križanac[3], Martina Šeruga Musić[1]

[1]*University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia;*
[2]*Leibniz Institute-DSMZ, German Collection of Microorganisms and Cell Cultures GmbH, Plant Virus Department, Braunschweig, Germany;*
[3]*Croatian Agency for Agriculture and Food, Centre for Plant Protection, Zagreb, Croatia*

dijana.skoric@biol.pmf.unizg.hr

Apple production is adversely affected by microbial diseases having diverse etiological agents including phytoplasmas, viruses and viroids. '*Candidatus Phytoplasma mali*' is agent of the devastating apple proliferation disease. Viruses belonging to the genera *Fovea*-, *Capillo*-, and *Trichovirus* are widespread and often harmful. Several viroids also negatively affect apple health, and novel ones whose effects on crop yield are still unknown are being discovered. One example is apple hammerhead viroid (AHVd). As apple is a woody perennial, mixed infections with different agents are a rule rather than an exception. Regardless, the impact of phytoplasmas, viruses and viroids on apple production has been insufficiently investigated in Croatia. We tested two asymptomatic apple fruit samples (MP1, MP2) collected in 2023 from central Croatia and 15 archival total nucleic acid extracts from leaf samples collected from commercial orchards in Northern and Eastern Croatia in 2011 and 2012 for the presence of AHVd by RT-PCR and Sanger sequencing. *'Ca. P. mali'* had been detected and genetically typed in the archival sample set previously. Four of the archival samples (392, 483, 489, 554) produced partial but informative Sanger sequences (respectively acc. nos. OQ921334, OR043662, OR043663 and OR043664), as well as the recently collected MP1 (OR043665) and MP2 (PV111791). Full-length AHVd genome sequences were confirmed from MP1 and MP2 by Illumina RNA sequencing as 433 nt-long sequence variants (PV130920, PV130921, PV130922), most closely related (98.62-97.70% nt identity) to AHVd isolate F1959 from Germany (PQ683290). Metagenomic analysis of MP1 and MP2 samples also revealed the occurrence of multiple viruses, namely apple stem pitting, apricot latent, apple green crinkle associated, apple stem grooving, apple chlorotic leaf spot, and citrus concave gum-associated virus. This confirms a long standing AHVd occurrence throughout the country in backyard apple trees and commercial orchards planted as early as 2000. Total nucleic acid extracts from leaves and fruits have proven to be suitable for AHVd detection by HTS and RT-PCR. Mixed infections with viruses and *'Ca. P. mali'* were also confirmed. These results provide a starting point for future research of multiple pathogens involved in apple infections in Croatia and their impact on apple production.

This work was supported by the by the Croatian Science Foundation (HRZZ) project NanoPhyto (grant no. IPS-2020-01-2960) and EU Horizon 2020 project European Virus Archive Global (EVAg, grant agreement no. 871029). This presentation was supported by the European Union – NextGenerationEU through the National Recovery and Resilience Plan 2021-2026 Institutional grant of the University of Zagreb Faculty of Science (AntroPOZ).

OP12

Microbiological Safety of Plant Based Dairy and Meat Substitutes at End of Shelf Life in Croatia

Jasenka Petrić, Brigita Hengl, Andrea Gross-Bošković, Barbara Lendić

Croatian Agency for Agriculture and Food, Center for Food Safety, Croatia

jasenka.petric@hapih.hr

In recent years, the European Union (EU) market has seen an increase in ready to eat (RTE) plant based dairy and meat substitutes. These products are formulated from diverse plant ingredients such as pulses, cereals, nuts and oilseeds, and are often produced using technologies that differ from conventional heat treated animal origin based products, and with different hygiene approaches. Such differences may influence to microbiological safety, including the survival of pathogens or their spores, as reported in recent studies detecting *Bacillus cereus* and *Clostridium* spp. in plant based ingredients or documented cases of *Listeria monocytogenes* contamination in RTE plant based food.

As part of an EFSA founded tailored made project involving 15 Member States, in 2025 101 RTE plant based dairy and meat substitute products available on the Croatian market were included. Samples were collected at retail, and microbiological testing was performed at the end of shelf life to assess the worst case scenario for microbial growth. The parameters examined included *Listeria monocytogenes*, *Listeria* spp., *Salmonella* spp., STEC, *E. coli*, spore forming bacteria (*Bacillus* spp. and *Clostridium* spp.), pH and water activity (a_w).

Although most products exhibited high a_w values (≥0.97) and pH levels supportive of microbial growth, *L. monocytogenes*, *Listeria* spp., *Salmonella* spp. and STEC were not detected in any sample. Hygiene indicator bacteria *E. coli* was detected in two tofu samples from the same producer (1.8×10^2 cfu/g). *Clostridium* spp. was not detected but *Bacillus* spp. was found in two samples, including one tofu product with a high bacteria count (3.5×10^5 cfu/g). The overall results indicate good microbiological safety of RTE plant based products available on the Croatian market. However, isolated detections of food safety indicator organisms in tofu products highlight the need for strengthened hygiene and process controls in minimally processed matrices. Nonetheless, the absence of major pathogens across all samples suggests that existing production hygiene practices are largely effective. This study provides relevant scientific support for ongoing European efforts to characterise the microbiological safety of RTE plant based food and to guide evidence based refinement of future risk management strategies.

OP13

Structural Genome Instability in *Deinococcus radiodurans* After Extreme Gamma-Irradiation

Filip Božić, Jelena Repar, Lucija Kumek, Davor Zahradka, Ksenija Zahradka

Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

fbozic@irb.hr

The bacterium *Deinococcus radiodurans* is renowned for its extraordinary resistance to ionizing radiation, surviving severe genome fragmentation through highly efficient RecA-driven double-strand break repair. However, *recA* mutants show residual repair via RecA-independent alternative end-joining; this enables cells to survive, but introduces large genomic deletions. Under extreme gamma-irradiation, even wild-type survivors accumulate faulty repair products. We hypothesized that irradiated cells ultimately failing to recover exhibit even higher frequency of such products. To investigate these genomic changes, wild-type and *recA* mutant populations were exposed to gamma-doses reducing survival by a factor of 10^6 to 10^7. Genomic DNA from the entire volume of irradiated populations was isolated at specific time points during recovery and analyzed using deep Illumina paired-end sequencing combined with computational structural variation analysis, including machine learning-based detection with Dysgu. In the *recA* mutant, radiation primarily caused uneven DNA synthesis and/or degradation, evidenced by marker frequency analysis, while structural variation patterns remained highly stochastic between replicates without clear directional trends. In contrast, the wild-type populations underwent active but highly error-prone DNA fragment reassembly, resulting in an increase in rearrangements during recovery. In these wild-type cells, deletions predominated during the early stages of recovery, whereas translocations become proportionally more frequent later on. Furthermore, while MFA indicated uniform DNA processing in the wild type, progressively increasing sequencing insert sizes suggested a continuous accumulation of complex structural rearrangements. Ultimately, extreme gamma-irradiation forces dying *D. radiodurans* populations into distinct genomic fates: while the *recA* mutant primarily shows asymmetric regional genome processing, wild-type cells engage in active but highly inaccurate and futile genome reassembly.

OP14

Alternative End-Joining Mechanism Contributes to the Repair of DNA Double-Strand Breaks in *Deinococcus radiodurans*

Jelena Repar, Davor Zahradka, Ksenija Zahradka

Ruđer Bošković Institute, Zagreb, Croatia

jrepar@irb.hr

The radioresistant bacterium *Deinococcus radiodurans* is renowned for its exceptional capacity to accurately reassemble its genome following hundreds of DNA double-strand breaks (DSBs). Typically, *D. radiodurans* relies on RecA protein to guide the high-fidelity DSB repair. However, structural changes rearranging the genome still occur, under intense radiation but also during the routine propagation and long-term storage of laboratory cultures. To understand how these genome rearrangements happen, we investigated the underlying DNA repair mechanisms.

We searched for genome rearrangements in both *recA* and wild type strains of *D. radiodurans* and characterized them using PacBio deep sequencing, *de novo* genome assembly and comparative genomics. In *recA* mutants, multiple rearrangements were found and identified as large deletions. Crucially, these deletions did not utilize longer direct repeats or insertion sequences (the typical substrates for the RecA-independent single-strand annealing previously found to repair DSBs in *D. radiodurans*), but instead rely on short (4–11 bp) direct repeats. Correspondingly, analysis of wild-type laboratory strains also revealed deletions between short (4–10 bp) repeats. Taken together, our findings indicate an operational alternative end-joining mechanism contributing to the repair of DSBs in *D. radiodurans*.

While alternative end-joining is well-known in eukaryotic organisms, its role in bacteria has been less clear. This error-prone mechanism probably serves as a backup system for processing dangerous DNA breaks in *D. radiodurans*, and might be important in shaping the bacterium's genome dynamics by driving structural variations and influencing its long-term evolution.

OP15

Artificial Intelligence-Enhanced FT-IR Spectroscopy for Rapid Serovar Identification of *Salmonella enterica* subsp. *enterica* Isolates from Croatia

Dora Tomašković[1], Maja Dopuđ[1], Sandra Šuto[2], Lucija Hlebić[1], Lovran Peinović[1], Silvio Špičić[1], Maja Zdelar Tuk[1], Irena Reil[1], Andrea Humski[1], Gordan Kompes[1], Silvija Šoprek Strugar[3], Bojan Papić[4], Jana Avberšek[4], Andrzej Mikolajczak[5], Miroslav Andrišić[1], Sanja Duvnjak[1]

[1]*Croatian Veterinary Institute, Zagreb, Croatia;*
[2]*Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia;*
[3]*University Hospital for Infectious Diseases Dr. Fran Mihaljević, Zagreb, Croatia;*
[4]*Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia;*
[5]*Bruker Daltonics GmbH & Co. KG, Bremen, Germany.*

peinovic@veinst.hr

Introduction: *Salmonella enterica* subsp. *enterica* remains one of the leading causes of foodborne bacterial infections worldwide and represents a major public health burden within the One Health framework. Rapid serovar identification is essential for epidemiological surveillance and outbreak investigations. Conventional serotyping based on the Kauffmann–White–Le Minor scheme is labour-intensive and dependent on extensive antisera collections. Fourier-transform infrared (FT-IR) spectroscopy combined with artificial intelligence (AI)-based analysis has emerged as a promising rapid alternative for bacterial characterisation. This study evaluated the discriminatory power of FT-IR spectroscopy using the IR Biotyper system (Bruker Daltonics, Germany) for differentiation of clinically relevant *S. enterica* serovars and its potential application in diagnostic microbiology.

Methods: A total of 143 *Salmonella enterica* isolates from human and food samples collected in Croatia were analysed using the IR Biotyper system. The collection included epidemiologically relevant serovars such as *S.* Enteritidis, *S.* Typhimurium and its monophasic variant, *S.* Infantis, *S.* Derby, *S.* Mbandaka, *S.* Hadar, and *S.* Coeln. Isolates were identified using MALDI-TOF MS prior to FT-IR analysis. Spectral data were divided into training and testing sets and analysed using AI-enhanced clustering, Support Vector Machines (SVM), and Artificial Neural Networks (ANN) integrated into the IR Biotyper software. Conventional serotyping according to HRN EN ISO/TR 6579-3:2014 was compared with FT-IR results.

Results: FT-IR spectroscopy demonstrated high reproducibility and strong discriminatory potential among analysed *Salmonella* strains. SVM and ANN algorithms enabled clustering according to O-serogroups and differentiation of

major serovars. Complete concordance with conventional serotyping was observed for predominant serovars included in the study. The method also showed high specificity and sensitivity while significantly reducing turnaround time compared with conventional workflows.
Conclusions: AI-enhanced FT-IR spectroscopy represents a rapid, reliable, and cost-effective approach for *Salmonella enterica* serovar differentiation. The method could improve laboratory surveillance and support faster detection of foodborne outbreaks through rapid strain characterisation. The study highlights the potential of FT-IR fingerprinting for AI-driven microbial characterisation.

OP16

Implementing a Synthetic Biology Approach to Unveil Gene Function Across Marine Microalgae (SynBioMM): Case-Study of the Vitamin B5 Biosynthesis in Coccolithophores

Frederic Chaux, Nensi Cuturic, Lucija Hrupec

Division for Marine and Environmental Research
Ruđer Bošković Institute, Croatia

fchaux@irb.hr

The "genetic dark matter" in phytoplankton:
The phytoplankton is the basic layer of marine ecosystems, yet it encompasses a rich diversity of photosynthetic microorganisms spread across the tree of life. Genomics and transcriptomics have become routine, producing vast catalogues of phytoplankton genes. However, their phylogenetic distance to well-studied eukaryotes prevents accurate prediction of gene function. In most phytoplankton clades, due to the lack of model organisms to support genetic approaches such as knock-in/knock-out, this “genetic dark matter” has become one of the main limitations in current phycology. To address this bottleneck, this project aims at characterising phytoplankton genes by heterologous expression in yeast, a highly practical eukaryotic model, notably by functional complementation (or rescue) of specific yeast mutants.
Case-study: B5 biosynthesis
To make a proof of concept, we studied a coccolithophore gene which is a putative fusion of two genes involved in the biosynthetic pathway of vitamin B5 (pantothenate). Preliminary experiments were conducted using published yeast deletion mutants *pan6Δ* and *ecm31Δ*, which were complemented by endogenous (yeast) genes, establishing the vector engineering and screening conditions appropriate for complementation assays. Similarly, we introduced truncated and full-length versions of the coccolithophore gene *panBC* to rescue yeast mutants in order to assess the function of PanB and PanC protein domains as well as the dual function of the gene fusion in vitamin B5 synthesis. Given the similarity of *panBC* to bacterial *panB* and *panC*, rather than to their eukaryotic counterparts, our functional characterization questions how prototrophy for B5 synthesis was acquired in this coccolithophore and related species.
SynBio: modularity, standardization and automation
Building upon the central concepts of synthetic biology, we built a fast-track pipeline to design PCR primers for direct cloning of phytoplankton cDNAs into yeast.

OP17

Uncovering hidden genetic diversity in the genus *Tobamovirus* through large-scale mining of public sequence repositories

Neža Pajek Arambašič[1], Katarina Bačnik[2], Luka Kranjc[2], Lana Vogrinec[2], Tomaž Curk[1], Denis Kutnjak[2]

[1]*University of Ljubljana, Faculty of Computer and Information Science, Slovenia*
[2]*National Institute of Biology, Slovenia*

neza.pajekarambasic@fri.uni-lj.si

The advancement of shotgun high-throughput sequencing has significantly accelerated RNA virus discovery, producing extensive datasets that often harbor unexplored viral sequences. Despite these technological gains, the large-scale reconstruction and taxonomic placement of viral genomes within specific groups remain hindered by fragmented assemblies and the limitations of fixed identity thresholds. We address these challenges by focusing on the genus *Tobamovirus* (family *Virgaviridae*), whose exceptionally stable virions are frequently detected across various environmental and biological matrices.

We employed a modular bioinformatic pipeline to mine global short-read repositories to discover yet-unknown tobamovirus diversity. Using the Serratus PalmID tool, we initially identified 253 candidate Sequence Read Archive (SRA) runs likely to contain novel tobamovirus sequences. These were processed through a reproducible Snakemake workflow for assembly and taxonomic filtering, yielding 510 candidate contigs. To streamline the identification process, we integrated expert curation with a supervised machine learning framework. This framework utilized an open reading frame (ORF) level Random Forest classifier based on 148 alignment-derived and intrinsic features, with ORF predictions aggregated into contig-level labels via stacked generalization.

Our methodology identified at least 32 putative novel tobamoviruses from diverse sources, including Amazon River freshwater metatranscriptomes, European municipal wastewater, soil, and various host-associated transcriptomes. Most novel discoveries clustered within established *Tobamovirus* subclades; however, several highly divergent sequences also formed distinct basal branches related to more divergent virgaviruses. These results emphasize the value of aquatic and wastewater systems as promising sample types for monitoring viral diversity. The scalable and modular framework developed here supports the continuous discovery of potential viruses as public sequencing data continues to grow.

SPONSORED LECTURE

SL1 IDEXX-sponsored lecture

From Colilert® to TECTA™: Advancing Microbiological Water Testing for Water and Wastewater Operations

Tomasz Marasek

IDEXX Laboratories, Poland

Tomasz-Marasek@idexx.com

This presentation highlights the evolution of rapid microbiological water testing, from Defined Substrate Technology (DST) methods such as Colilert®, Enterolert®, and Pseudalert® to automated real-time platforms such as TECTA™. These technologies have improved the speed and reliability of detecting *E. coli*, total coliforms, and enterococci through standardized workflows widely adopted in the water sector.

The presentation will focus on the practical value of rapid microbiological testing for water and wastewater operators, research institutions, and organizations without dedicated microbiology laboratories. By providing fast on-site results with minimal hands-on time and training requirements, automated testing enables earlier detection of contamination events and faster operational decision-making.

Particular attention will be given to applications supporting bathing water safety, where rapid detection of *E. coli* and intestinal enterococci can help authorities identify contamination sooner, protect public health, and make more informed decisions during the bathing season. The presentation will also discuss the role of automated microbiological monitoring in drinking water, wastewater, environmental waters, and emergency response situations, where reducing the time between sampling and actionable results can significantly improve water quality management and public health protection.

IDEXX Water Testing Solutions is a recognized expert in microbiological water quality testing, providing innovative technologies that help laboratories and water operators detect indicator microorganisms quickly, accurately, and efficiently. IDEXX solutions, including Colilert®, Enterolert®, Pseudalert®, Legiolert®, EasyDisc® and the automated TECTA™ platform, are used worldwide to support regulatory compliance and protect public health. Through continuous innovation and scientific expertise, IDEXX helps water professionals make faster, more informed decisions to ensure safe drinking water and environmental water quality.

CROSS-DISCIPLINARY MODULES

CDM1

AI-driven Microbiology

Tomaž Curk

University of Ljubljana, Faculty of Computer and Information Science, Slovenia

tomaz.curk@fri.uni-lj.si

How can artificial intelligence be meaningfully integrated into microbiological research?

This two-hour cross-disciplinary module will explore both machine-learning approaches for analysing biological data and the rapidly expanding use of generative AI in scientific work. Starting from the pre-congress survey of participants' current practices and needs, the session will combine a conceptual introduction, a case study from microbiological research, practical examples of accessible AI tools, and discussion of their limitations, reliability, reproducibility and responsible use.

The emphasis will be on helping experimental microbiologists understand where AI approaches can be useful and how to evaluate them critically, rather than on technical or mathematical detail.

CDM2

Microbiology Meets Industry

Iva Guberović

Nuqleus Innovation Centre Nikola Tesla, Croatia

iva.guberovic@icent.hr

How does microbiological research move from the laboratory to real-world application? This cross-disciplinary module explores different pathways for translating discoveries in microbiology into technologies, startups and market-ready products.

The session will combine an introduction to technology transfer and commercialization with real-life examples. Dragomira Majhen (VirusHunter) will share experience from building a microbiology-based startup, while Martin Kavšček (Acies Bio) will provide an industry perspective on developing and scaling microbial technologies from laboratory concepts towards industrial biomanufacturing.

The module will conclude with an interactive panel discussion on key challenges and opportunities in moving microbiological research towards industrial application. Egon Bech Hansen will join the panel, bringing an international perspective from applied and industrial biotechnology.

CDM3

Microbiology Going Public

Petra Buljević Zdjelarević, Marko Košiček

Ruđer Bošković Institute, Croatia

buljevic@irb.hr, mkosicek@irb.hr

How can the same microbiological topic be communicated effectively to very different audiences? This interactive module focuses on translating scientific evidence into clear, accurate and audience-appropriate messages for the public, the media and policymakers.

The module will begin with an introduction by Petra Buljević Zdjelarević and Marko Košiček, providing background on key principles and challenges of science communication. This will be followed by two short TED-style talks: Ana Bielen – My Journey into Communicating Microbiology to Children and Ines Sviličić Petrić – How Do You Make the Invisible Matter? Explaining science in a language people can act on.

Participants will then work in small groups to adapt the same microbiological topic to different communication settings: explaining it to children and families at a public outreach event, responding to media questions, or briefing decision-makers on its relevance and possible next steps.

Particular attention will be given to communicating risk and uncertainty, distinguishing evidence from interpretation, avoiding both unnecessary alarm and false reassurance, and identifying what different audiences need to know.

WORKSHOPS

WS1

Meet The Editor: Navigating The Scientific Publishing Process

Iva Grabarić Andonovski

Food Technology and Biotechnology

iva.grabaric.andonovski@pbf.unizg.hr

Publishing a scientific paper involves much more than preparing a manuscript. This interactive workshop will guide participants through key stages of the scientific publishing process, from selecting an appropriate journal and preparing a submission to navigating peer review, responding to reviewers, publication ethics and communication with editors.

WS2

Orange Data Mining Workshop

Tomaž Curk

University of Ljubljana, Faculty of Computer and Information Science, Slovenia

tomaz.curk@fri.uni-lj.si

This 90-minute workshop will introduce participants to data exploration and machine learning using Orange Data Mining, a visual data-analysis platform that allows users to build and explore analytical workflows without programming. Participants will work directly with data on their own laptops and will be introduced to selected basic concepts and workflows through practical examples.

The workshop is intended for microbiologists with little or no previous experience in machine learning or data science; no programming skills are required.

POSTER PRESENTATIONS

P1

Age Distribution and Type-Specific Circulation of Respiratory Syncytial Virus Among Children in Croatia During the 2025/2026 Season

Dragan Jurić, Ilijana Stojković, Anita Jurić, Lara Samadan Marković, Željka Hruškar, Marija Marković, Ivana Ferenčak, Irena Tabain

Croatian Institute of Public Health, Zagreb, Croatia

dragan.juric@hzjz.hr

Respiratory syncytial virus (RSV) remains one of the leading viral causes of acute respiratory tract infections, particularly among infants and young children. Understanding seasonal RSV activity and the distribution of circulating virus types is important for monitoring disease burden and guiding preventive strategies. We analysed laboratory-confirmed RSV infections detected during the 2025/2026 season in Croatia.

Between epidemiological week 40/2025 and week 20/2026, a total of 797 RSV-positive patients were identified by real-time polymerase chain reaction (RT-PCR) using the Respiratory Viruses 16-Well Panel (AUS Diagnostics, Australia). RSV typing was available for 676 cases, of which 368 (54.4%) were RSV-A and 308 (45.6%) RSV-B. Viral activity increased during winter, reaching the highest number of detections in week 9/2026, with 137 RSV-positive cases. High activity was observed mainly between weeks 6 and 10/2026, when weekly detections ranged from 75 to 137 cases.

Small children represented the overwhelming majority of RSV cases. Patients aged 0–10 years accounted for 660/797 (82.8%) detections, while only 137 cases were recorded in older age groups. The highest number of infections was observed among children aged 12–24 months (196 cases), followed by those aged 2–5 years (183 cases), infants aged 0–5 months (156 cases), and infants aged 6–11 months (137 cases). Together, children younger than five years accounted for 672 cases (84.3% of all detections). Among typed paediatric samples, RSV-A and RSV-B co-circulated throughout the season, with RSV-A accounting for 362 cases (54.8%) and RSV-B for 298 cases (45.2%).

The 2025/2026 RSV season was characterised by substantial viral activity and a pronounced burden among young children, particularly those under five years of age. Although both RSV types circulated concurrently, RSV-A showed a slight predominance. Continuous RSV surveillance remains essential for monitoring seasonal trends, detecting shifts in subtype distribution, and supporting future prevention strategies targeting populations at highest risk.

P2

Insights Into Human Adenovirus Type 26-Induced Inflammasome Activation: Impact of Clathrin-Mediated Endocytosis

Ana Runtić, Lucija Lulić Horvat, Isabela Drašković, Dragomira Majhen

Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

aruntic@irb.hr

Human adenoviruses are non-enveloped, double-stranded DNA viruses that generally cause mild infections. They are employed as vectors in gene therapy and vaccine research, especially for low-seroprevalence types such as human adenovirus type 26 (HAdV-26), which has been extensively studied and used in numerous clinical trials. However, the activation of innate immunity by HAdV-26 in epithelial cells has not been fully examined. The inflammasome is a multiprotein signalling complex promoting the activation of pro-inflammatory interleukins, such as IL-1β and IL-18, via caspase-1, which is vital for the inflammatory response. This study aimed to investigate how inhibiting clathrin-mediated endocytosis (CME) affects inflammasome activation in human lung carcinoma cells (A549) infected with HAdV-26. Alongside A549 cells we used the A549-E6 clone, with increased $\alpha_V\beta_3$ integrin expression, for which we reported to be prerequisite for efficient transduction of epithelial cells with HAdV-26. Using the RT^2 Profiler PCR Array, we observed increased expression of inflammasome-related genes in both cell lines following infection and after CME inhibition, as confirmed by additional quantitative PCR performed 1 hour post-infection. The HAdV-26 infection decreased gene expression of IL-18, and caspase-1 in both cell lines, except for IL-1β in A549 cells, which shows an increase. In contrast, CME inhibition in HAdV-26-infected cells increased the expression of these genes. Inflammasome activation was further analysed at the protein level by immunofluorescence and confocal microscopy at 3 and 24 hours post-infection. We did not observe significant changes in the subcellular localization or colocalization of the inflammasome-forming proteins ASC and NLRP3 in both experimental setups. However, cathepsin B showed increased cytoplasmic dispersion upon infection, which was amplified by CME inhibition. Results suggest a synergistic effect of HAdV-26 infection and CME inhibition on the regulation of inflammasome-related gene expression. Additionally, the lysosomal release of cathepsin B may signal the assembly and activation of the inflammasome. Overall, the findings provide instructive evidence of inflammasome activation in HAdV-26-infected cells, but additional experiments are needed to clarify the temporal regulation of these processes and the impact of CME in HAdV-26 infection. Advancements in this field will aid in the development of improved adenoviral vectors.

P3

First Ceftriaxone-Resistant Extensively Drug-Resistant *Neisseria gonorrhoeae* in Croatia

Ivana Bešlić[1], Dragan Jurić[1], Tatjana Nemeth Blažić[1], Nilia Volarević[2], Daniela Glažar Ivče[2], Ivana Ferenčak[1], Ljiljana Žmak[1,3], Dolores Peruč[2], Susanne Jacobsson[4], Daniel Golparian[4], Ivana Božičević[5], Iskra Alexandra Nola[6], Magnus Unemo[4,7], Tajana Juzbašić[1]

[1]*Croatian Institute of Public Health, Zagreb, Croatia*
[2]*Teaching Institute of Public Health of the Primorje-Gorski Kotar County, Rijeka, Croatia*
[3]*School of Medicine, University of Zagreb, Zagreb, Croatia*
[4]*WHO Collaborating Centre for Gonorrhea and Other STIs, National Reference Laboratory for STIs, Faculty of Medicine and Health, Örebro University, Örebro, Sweden*
[5]*WHO Collaborating Centre for HIV Strategic Information, Andrija Štampar School of Public Health, School of Medicine, University of Zagreb, Zagreb, Croatia*
[6]*Andrija Štampar School of Public Health, School of Medicine, University of Zagreb, Zagreb, Croatia*
[7]*Institute for Global Health, University College London, London, United Kingdom*

dragan.juric@hzjz.hr

We report the first laboratory-detected case of extensively drug-resistant *Neisseria gonorrhoeae* (XDR-NG) displaying resistance to ceftriaxone and high-level azithromycin resistance (HLAzir) in Croatia.

At the time, parenteral ceftriaxone combined with oral azithromycin constituted first-line dual therapy for gonorrhea. Since 2018, XDR *Neisseria gonorrhoeae* strains combining ceftriaxone resistance with HLAzir have been sporadically detected in Europe and other regions, with a high prevalence reported in some Asian countries, including Cambodia. In the 2024 European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP), two ceftriaxone-resistant *N. gonorrhoeae* isolates were identified in the EU/EEA, both of which were classified as XDR. Until the present case, ceftriaxone-resistant XDR gonorrhea had not been reported in Croatia.

In February 2026, a heterosexual man reported unprotected sexual intercourse with an unknown female in Croatia, who was not possible to trace. Three days later, he presented to a general practitioner with dysuria and was empirically treated with amoxicillin–clavulanic acid (1 g twice daily for 7 days). Due to persistent symptoms and appearance of urethral discharge, a urethral swab was collected on day 14, yielding positive result for NG on NAAT and culture (isolate HR027). Antimicrobial susceptibility testing of HR027 showed HLAzir, resistance to ceftriaxone, cefixime, tetracycline, ciprofloxacin and benzylpenicillin, as well as penicillinase production. The patient was successfully treated with intravenous 2 g ceftriaxone.

Whole-genome sequencing (WGS) identified the mosaic *penA*-60.001 allele and 23S rRNA A2045G mutation associated with ceftriaxone/cefixime and HLAzir, respectively. The isolate was assigned to MLST ST9903 and the novel NG-STAR ST7905 (NG-STAR CC7557). Comparative genomic analysis placed HR027 within genomic lineage B, distinct from the previously described Cambodian XDR-NG clone in genomic lineage A.

The detection of XDR *N. gonorrhoeae* in Croatia highlights the growing international threat posed by strains combining ceftriaxone resistance associated with *penA*-60.001 and HLAzir. Strengthened gonorrhea prevention, timely diagnosis, treatment and reporting, together with integrated antimicrobial resistance and genomic surveillance through the Euro-GASP programme, are essential for early detection of emerging resistance and guiding effective treatment strategies.

P4

Indoor Dust Microbiome as a Mediator Between Environment and Child Immune Health: Insights from the Zagreb EDIAQI Pilot Study

Iva Šunić[1], Dubravka Havaš Auguštin[1], Jelena Šarac[1,2], Kristina Michl[3,4], Tomislav Cernava[3,5], Mario Lovrić[1,2,6]

[1]*Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb, Croatia*
[2]*Faculty of Biotechnology and Drug Development, University of Rijeka, Rijeka, Croatia*
[3]*Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria*
[4]*Department Life Science Engineering, University of Applied Sciences Technikum Wien, Vienna, Austria*
[5]*School of Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton, Southampton, United Kingdom*
[6]*Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia*

iva.sunic@inantro.hr

Indoor environments represent the primary habitat for modern humans, yet the microbial communities inhabiting indoor dust remain poorly characterized in the context of child health. This study, conducted within the Horizon Europe EDIAQI project, aimed to investigate the composition and diversity of the indoor dust microbiome and its potential links to respiratory health outcomes, particularly asthma, in children. Dust samples were collected from households participating in the Zagreb pilot project and subjected to amplicon sequencing on Illumina MiSeq targeting the bacterial 16S rRNA gene and the fungal ITS region. Bioinformatic pipelines were applied to characterise microbial community structure across samples. Beta diversity analysis (PERMANOVA) revealed statistically significant differences in microbial community composition across households. For the bacterial microbiome, the strongest predictors were pet presence in the household ($p < 0.001$), child's gender, and AC cleaning frequency (both $p< 0.001$), followed by number of siblings and living environment (all $p < 0.01$). For the fungal microbiome significant drivers were house occupants, cat presence ($p<0.001$) and siblings ($p < 0.01$), and cleaning practices such as dusting, curtains and carpets cleaning- all $p < 0.05$. Several bacterial genera, including *Streptococcus*, *Neisseria*, and *Prevotella*, and fungal taxa including *Cladosporium* and *Alternaria* were identified as differentially abundant across household types including pet presence, occupancy, living environment, and cleaning habits. These findings suggest that indoor dust microbiome composition is shaped by a complex
interplay of household and lifestyle factors, and may play a role in modulating children's immune responses via the hygiene hypothesis. The diversity and

composition of microbial communities in indoor dust may play a key role in early immune education. Our results indicate that everyday household decisions, from how frequently surfaces are dusted to whether a cat is kept indoors, may meaningfully shape the microbial landscape to which children are exposed, with potential downstream consequences for immune development and respiratory health.

P5

Development of a HIEC-6-Based *in vitro* Model for Studying SIBO-Associated Host–Microbe Interactions

Aleksander Mahnič[1,2], Maja Rupnik[1,2], Lidija Gradišnik[2], Uroš Maver[2], Kaja Tominc[1], Sabina Mlakar[1], Jernej Vajda[2], Boštjan Vihar[2]

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

maja.rupnik@nlzoh.si

Small intestinal bacterial overgrowth (SIBO) is associated with altered microbial load and host–microbe interactions in the small intestine, yet *in vitro* models often rely on cancer-derived epithelial cell lines with limited physiological relevance. This study aimed to develop an improved SIBO-relevant gut model using the non-transformed HIEC-6 intestinal epithelial cell line and to evaluate its response to bacterial stimuli.

HIEC-6 cells were exposed to heat-inactivated *Escherichia coli* cultures at concentrations representing physiological (10^2) and SIBO-like (10^5) conditions, alongside a no-bacteria control. Cells were matured prior to treatment, after which inactivated bacteria were added on days 0, 1, and 3. Samples were collected on days 1, 3, and 5. Cellular responses were assessed using MTT assays for metabolic activity, microscopy for morphology, and RNA sequencing following mRNA enrichment for differential gene expression analysis.

Metabolic activity and cellular morphology were not significantly affected by exposure to inactivated *E. coli* at either concentration. Transcriptomic analysis revealed that gene expression patterns were predominantly driven by cell culture age (days 1, 3, and 5). Differential expression analysis (DESeq2) further indicated that responses associated with bacterial exposure were not gradual across concentrations but instead showed distinct profiles between 10^2 and 10^5 conditions. Notably, these differences remained secondary to the strong temporal effects.

Overall, HIEC-6 cells exhibit stable metabolic responses under SIBO-like bacterial exposure, while transcriptional changes are primarily influenced by cell maturation state. This model provides a promising platform for studying host–microbe interactions under controlled, physiologically relevant conditions, though careful consideration of temporal dynamics is essential.

P6

Endometrial Cancer Is Not Associated with Distinct Microbiota Signature but Shows Tumour-Enriched Cervix-Associated Taxa

Zala Vipotnik[1], Živa Poberžnik[1], Maja Pakiž[2], Sabina Mlakar[1,3], Maja Rupnik[1,3], Aleksander Mahnič[1,3]

[1]*Faculty of Medicine, University of Maribor, Maribor, Slovenia*
[2]*University Clinical Centre Maribor, Maribor, Slovenia*
[3]*National Laboratory for Health, Environment and Food, Maribor, Slovenia*

maja.rupnik@nlzoh.si

The role of the endometrial microbiota in endometrial cancer remains poorly understood, partly due to the low-biomass nature of the uterine environment and potential microbial transfer from adjacent anatomical sites. This study aimed to characterize the endometrial microbiota in patients with endometrial cancer compared to controls, and to assess potential microbial reservoirs contributing to its composition.

We performed 16S rRNA gene sequencing on endometrial samples from 17 patients with endometrial cancer and 16 control patients with uterine prolapse. Multiple anatomical regions of the endometrium were sampled using both swabs and biopsies. To address the high risk of contamination inherent to low-biomass samples, we included an extensive set of negative controls. In addition, cervical and anorectal swabs were collected to evaluate potential extra-uterine sources of the endometrial microbiota.

The endometrial microbiota showed low prevalence and marked heterogeneity, both between individuals and across anatomical regions within the same individual. When microbial composition was more homogeneous across endometrial regions of a given patient, this pattern was associated with increased similarity to the cervical microbiota, suggesting enhanced microbial translocation from the cervix. No cancer-specific taxonomic signatures or major shifts in overall microbial community structure were detected in patients with endometrial cancer as compared to control patients. However, in cancer patients, the microbiota present directly on tumor tissue contained a higher proportion of cervical-associated taxa compared with other endometrial regions from the same patient.

These findings suggest that endometrial cancer is not associated with a distinct endometrial microbial signature, but tumor tissue may be more permissive to colonization by microbes originating from the cervix.

P7

Molecular Characterisation Reveals Sex-Associated Distribution of Dermatophytes Causing Onychomycosis in Croatia

Ida Ćurtović Pučar[1], Marta Kozarić[1], Ana Čičmak[2], Danka Jovetić[3], Mario Sviben[2], Maja Šegvić Klarić[1], Sanja Dabelić[1], Daniela Jakšić[1]

[1]*Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia*
[2]*Breyer Medical Diagnostics, Zagreb, Croatia*
[3]*Croatian Institute of Public Health, Zagreb, Croatia*

daniela.jaksic@pharma.unizg.hr

Background. Onychomycosis is the most prevalent nail disorder, with dermatophytes as the principal aetiological agents. Routine identification relies mainly on culture morphology, which may be slow and diagnostically challenging. We characterised dermatophytes causing onychomycosis in a Croatian cohort by ITS sequencing and compared molecular identification with routine culture-based assessment.

Methods. Dermatophyte isolates from nail specimens collected between 2021 and 2023 were identified by culture morphology and sequencing of the internal transcribed spacer (ITS) region. Species assignment was performed against reference type strains, including *T. rubrum*, the *T. mentagrophytes* complex and *T. tonsurans*, using Maximum Likelihood phylogeny under the GTR+Γ model. A subset of *T. rubrum* isolates was additionally analysed at the β-tubulin (*BT2*) locus. The analysis included 128 nail isolates with both morphological and molecular identification.

Results. The cohort comprised 128 patients, 65 men and 63 women, with a median age of 58 years, range 16–87. ITS sequencing identified *T. rubrum* as the predominant species, detected in 82/128 isolates (64%), followed by the *T. mentagrophytes* complex in 45/128 (35%) and *T. tonsurans* in one isolate (1%). Culture and ITS results were concordant in 123/128 isolates (96%). The five discordant isolates (4%) had been assigned morphologically to the *T. mentagrophytes* complex but were identified as *T. rubrum* by ITS. *T. rubrum* predominated in men (50:32), whereas the *T. mentagrophytes* complex was more frequent in women (15:30); this sex-associated species distribution was significant ($\chi^2 = 7.8$, $p = 0.005$; $OR = 3.1$). Most specimens were toenails (124/128). Sequenced *T. rubrum* isolates showed minimal variability at ITS and *BT2* loci, consistent with a near-clonal local population.

Conclusion. *T. rubrum* was the leading cause of dermatophyte onychomycosis in this Croatian cohort and showed a highly conserved molecular profile. ITS sequencing corrected approximately 4% of culture-based identifications, supporting its diagnostic value. The significant sex-related species distribution

warrants further investigation. Species-level resolution within the *T. mentagrophytes* complex, including discrimination of potentially terbinafine-resistant *T. indotineae*, will require additional markers such as *BT2* and *TEF1-α*.

P8

Diversity, Distribution, and Antifungal Susceptibility of *Neocosmospora* species Within the *Fusarium solani* Species Complex

Ida Ćurtović Pučar[1], Marta Kozarić[1], Daniela Jakšić[1], Sanja Pleško[2], Marko Siroglavić[2], Zrinka Bošnjak[2], Cornelia Lass-Flörl[3], Martin Hoenigl[4], Maja Šegvić Klarić[1]

[1]*University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia*
[2]*University Hospital Zagreb, Zagreb, Croatia*
[3] *Institute of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria*
[4] *Division of Infectious Diseases, Department of Internal Medicine, Medical University of Graz, Graz, Austria*

maja.segvic@pharma.unizg.hr

The *Fusarium solani* species complex (FSSC) represents a heterogeneous group of opportunistic fungi of increasing significance in clinical contexts. This study examined their distribution among FSSC and compared antifungal susceptibility across species.

A total of 95 FSSC environmental (drains, washing machines, bananas and cornmeal) and clinical isolates from Croatia and Austria were included in the study. Identification was performed using MALDI-TOF MS and sequencing ITS, *tef1α*, and *rpb2* gene regions. Antifungal susceptibility testing to amphotericin B (AMB), voriconazole (VOR), posaconazole (POS), isavuconazole (ISA), olorofim (OLF), and manogepix (MGX) was conducted according to CLSI guidelines.

N. keratoplastica (*n*=28) and *N. petroliphila* (*n*=24) were dominant species, followed by *N. solani* (*n*=14), *N. tonkinensis* (*n*=9), *N. falciformis* (*n*=5), *N. metavorans* (*n*=5), *N. crassa* (*n*=3), *N. gamtoosensis*/*N. solani* (*n*=3), *N. stericola* (*n*=2), *N. quercicola* (*n*=1), and *N. pisi* (*n*=1). Clinical strains *of N. keratoplastica* (*n*=12) were primarily recovered from respiratory and wound samples, whereas *N. petroliphila* (*n*=19) was predominantly isolated from stool, blood, urine and respiratory samples. Environmental isolates of both species were predominantly obtained from drains. Sequencing enabled reliable species-level identification, while MALDI-TOF MS correctly assigned isolates at the FSSC complex level.

FSSC isolates showed high MIC values for POS and ISA (≥16 mg/L) and generally elevated VOR MICs (16 to >16 mg/L). AMB MICs ranged from 0.5–8 mg/L, with few isolates (3/95) reaching ≥16 mg/L. OLF demonstrated limited in vitro activity (MIC ≥16 mg/L). In contrast, MGX showed potent activity with low MEC values across many isolates. Most FSSC isolates (60/95) exhibited low MECs (≤0.03–0.06 mg/L), particularly *N. petroliphila*, *N. falciformis*, and a subset of *N. keratoplastica*, while

16 isolates showed elevated values (8 to >16 mg/L), indicating heterogeneous antifungal susceptibility across the complex.
The presence of FSSC in clinical and environmental samples, together with reduced azole susceptibility, underscores its clinical importance and persistence. Notably, *N. keratoplastica* and *N. petroliphila* were frequently recovered from both sources. MGX showed promising antifungal activity, highlighting its potential for future treatment of FSSC infections.

The research was funded by the Croatian Science Foundation (HRZZ-IP-2022-10-8062).

P9

Ecological Modulation of Multispecies Oral Biofilms by *Streptococcus salivarius*

Gabrijela Begić[1], Ljerka Karleuša Mujkić[2], Olga Cvijanovic Peloza[3], Ivana Gobin[1]

[1]*Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*
[2]*Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*
[3]*Department of Anatomy, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*

gabrijela.begic@uniri.hr

Background: Oral biofilms are complex microbial communities whose composition and interactions determine the transition between health and disease. Increasing attention has been directed toward probiotics as tools for oral microbiome modulation and restoration of ecological balance within biofilms. *Streptococcus salivarius* is considered one of the most promising probiotic candidates.
Objective: To investigate the influence of *Streptococcus salivarius* on the composition, structure, and architecture of multispecies oral biofilms.
Methods: An *in vitro* multispecies oral biofilm consisting of *Streptococcus mutans*, *Streptococcus oralis*, and *Aggregatibacter actinomycetemcomitans* was established in the presence and absence of *Streptococcus salivarius*. Biofilm composition was evaluated using culture-based methods, while biofilm structure and organization were analyzed by CLSM and SEM.
Results: The addition of *Streptococcus salivarius* altered biofilm composition, resulting in reduced proportions of *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans*. CLSM demonstrated thinner biofilms with reduced EPS content, while SEM confirmed a simpler and less complex biofilm architecture.
Conclusion: *Streptococcus salivarius* modulated the composition and structure of multispecies oral biofilms without complete eradication of the microbial community.

P10

hLF1-11 as a Colistin-Sparing Adjuvant Against Multidrug-Resistant *Acinetobacter baumannii*: *in vitro* Synergy and Antibiofilm Activity

Ivana Lakošeljac[1], Ivana Gobin[1,2], Dolores Peruč[1,2], Željko Linšak[1,2], Davorka Repac Antić[2,3]

[1]*Teaching Institute of Public Health of Primorsko-Goranska County, Rijeka, Croatia*
[2]*Faculty of Medicine, University of Rijeka, Rijeka, Croatia*
[3]*Clinical Hospital Center Rijeka, Rijeka, Croatia*

ivana.lakoseljac@zzjzpgz.hr

Objective: Multidrug-resistant *Acinetobacter baumannii* represents a major therapeutic challenge. Colistin remains a last-resort therapeutic option, but its use is limited by toxicity and emerging resistance. hLF1-11, a human lactoferrin-derived antimicrobial peptide, has shown potential as an adjunctive agent. This study evaluated the hLF1-11–colistin interaction against *A. baumannii* and the effect of synergistic combinations on biofilm formation.
Methods: Two strains were tested: *A. baumannii* ATCC BAA-1605 and a multidrug-resistant clinical isolate (MDR-2900). Minimum inhibitory (MIC) and bactericidal (MBC) concentrations were determined by broth microdilution (EUCAST). Synergy was assessed by checkerboard assay (77 combinations per strain; colistin 0.00098–1 mg/L × hLF1-11 8–512 mg/L for ATCC BAA-1605, colistin 0.03125–2 mg/L × hLF1-11 0.5–512 mg/L for MDR-2900). Fractional inhibitory concentration indices (FICi) were calculated, with FICi ≤ 0.5 indicating synergy. Biofilm inhibition on polystyrene was evaluated by crystal violet staining.
Results: Colistin MICs were 0.5 and 1.0 mg/L, respectively; the hLF1-11 MIC was 512 mg/L for both strains. Synergy occurred in 8/77 combinations for ATCC BAA-1605 (minimum FICi 0.188, colistin 0.03125 + hLF1-11 64 mg/L) and 7/77 for MDR-2900 (minimum FICi 0.250, colistin 0.125 + hLF1-11 64 mg/L), i.e. 16-fold and 8-fold colistin MIC reductions at one-eighth of the hLF1-11 MIC. At 256 mg/L hLF1-11, ATCC BAA-1605 was inhibited at colistin 0.00098 mg/L (512-fold reduction), although such combinations were additive rather than synergistic. The colistin MBC for ATCC BAA-1605 fell 4-fold (1 to 0.25 mg/L) with hLF1-11 32 mg/L; for MDR-2900 the bactericidal and inhibitory endpoints coincided. No antagonism was detected. Sub-inhibitory combinations markedly reduced biofilm formation, whereas either agent alone at sub-inhibitory concentrations increased it.
Conclusions: hLF1-11 enhanced the in vitro activity of colistin against *A. baumannii*, substantially reducing inhibitory and bactericidal concentrations and enabling bactericidal activity at sub-MIC colistin. Selected sub-inhibitory combinations markedly reduced biofilm formation, suggesting benefits beyond direct antimicrobial activity. These findings support further development of hLF1-

11 as an adjunctive strategy for preserving colistin efficacy against multidrug-resistant *A. baumannii*. Validation in a larger collection of clinical isolates is underway.

P11

Modulation of Biofilm Formation and Virulence Determinants in *Pseudomonas aeruginosa* by Essential Oils of *Micromeria thymifolia* (Scop.) Fritsch and *Calamintha officinalis* Moench

Gordana Maravić-Vlahoviček[1], Marija Kindl[2], Dora Koprivčić[1], Iva Sičić[1], Sonja Obranić[3], Sanda Vladimir-Knežević[2]

[1]*Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia*
[2]*Department of Pharmacognosy, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia*
[3]*Department of Nursing, University Centre Varaždin, University North, Varaždin, Croatia*

gordana.maravic@pharma.unizg.hr

Pseudomonas aeruginosa is a major cause of hospital-acquired infections worldwide and is associated with high morbidity and mortality rates. The treatment of *P. aeruginosa* infections is complicated by its intrinsic, acquired, and adaptive resistance to antibiotics. Therefore, alternative strategies targeting bacterial virulence and quorum sensing systems are increasingly being explored to improve the efficacy of conventional antimicrobial therapies. The aim of this study was to investigate, for the first time, the effects of essential oils from *Micromeria thymifolia* (Scop.) Fritsch and *Calamintha officinalis* Moench on quorum sensing-regulated virulence factors and biofilm formation in *P. aeruginosa*.

Antibiofilm activity was assessed by exposing bacterial cultures to subinhibitory concentrations of essential oils (MICs: 4 mg/mL for *M. thymifolia* and 6 mg/mL for *C. officinalis*) in 96-well microtiter plates. Biofilm biomass was quantified using crystal violet staining and absorbance measurement at 540 nm. Swarming motility was evaluated on LB agar supplemented with 0.5 mg/mL essential oil, while proteolytic activity was determined on skim milk agar. Pyocyanin production was measured in cultures grown in LB medium containing 0.5 mg/mL essential oil, followed by extraction and spectrophotometric quantification at 520 nm. Results were normalized to bacterial growth.

Both essential oils inhibited the growth of planktonic *P. aeruginosa* cells, with *M. thymifolia* active at concentrations of 1–2 mg/mL and *C. officinalis* at 0.5–4 mg/mL. In addition, both oils significantly reduced biofilm formation and swarming motility, indicating interference with quorum sensing-regulated behaviors. Neither essential oil affected proteolytic activity. However, *M. thymifolia* significantly decreased pyocyanin production, whereas *C. officinalis* showed no effect on this virulence factor.

These findings demonstrate that the investigated essential oils possess both antimicrobial and anti-virulence properties against *P. aeruginosa*. Their ability to inhibit biofilm formation and quorum sensing-associated phenotypes highlights their potential as adjunctive agents for the treatment of infections caused by this clinically important pathogen. Further studies are warranted to elucidate their mechanisms of action and therapeutic applicability.

P12

Antimicrobial and Antibiofilm Activity of Selected Natural Compounds Against *Legionella pneumophila* Under Nutritive and Environmental Conditions

Marina Pribanić Matešić, Patrik Mudri, Roko Maraš, Ljerka Karleuša Mujkić, Ivana Gobin

Faculty of Medicine, University of Rijeka, Rijeka

ivana.gobin@uniri.hr

Introduction. *Legionella pneumophila* is an opportunistic waterborne pathogen capable of persisting in engineered water systems through adaptation to environmental stress and biofilm formation. The persistence of Legionella within biofilms contributes to the limited efficacy of conventional disinfection strategies and promotes long-term colonization of water distribution systems. Natural compounds have attracted increasing attention as environmentally friendly antimicrobial agents with potential applications in water safety management. This study aimed to evaluate the antimicrobial activity of selected natural compounds against planktonic and biofilm-associated *L. pneumophila* under both nutritive and environmental conditions.

Materials and Methods. The antimicrobial activity of citric acid, acetic acid, carvacrol, α-pinene and linalool was evaluated against *L. pneumophila*. Minimum inhibitory (MIC) and bactericidal (MBC) concentrations were determined in Buffered Yeast Extract (AYE) medium, while the minimum effective concentration (MEC) was established in Legionella Survival Medium (LSM). The effects of the tested compounds on planktonic cells were additionally assessed using LIVE/DEAD fluorescence staining. Antibiofilm activity was evaluated by treating biofilms with MIC, MBC and sub-MIC concentrations followed by viable cell enumeration.

Results. The tested natural compounds exhibited variable antimicrobial activity depending on the experimental conditions. Acetic and citric acids demonstrated MIC values of 0.5% in AYE, while lower MEC values were observed in LSM (0.06125% and 0.125%, respectively). Among the plant-derived compounds, α-pinene exhibited the greatest activity in AYE, whereas carvacrol demonstrated the lowest MEC in LSM. LIVE/DEAD staining confirmed reduced viability of planktonic cells following treatment with the tested compounds. In addition, all compounds reduced the number of viable biofilm-associated cells, although their efficacy varied according to the compound and experimental conditions.

Conclusions. Selected natural compounds demonstrated antimicrobial activity against both planktonic and biofilm-associated *L. pneumophila* under nutritive

and environmental conditions. The combined microbiological, fluorescence and antibiofilm findings suggest that natural compounds may represent promising complementary tools for controlling Legionella survival and biofilm formation in water systems.

P13

Surface-Dependent Efficacy of UVC Irradiation Against 24-Hour *Candidozyma auris* Biofilms

Marina Pribanić Matešić[1], Ema Poljak[1], Ivana Jelovica Badovinac[2], Ivana Gobin[1]

[1]*Faculty of Medicine, University of Rijeka, Department of Microbiology and Parasitology, Rijeka, Croatia*
[2]*Faculty of Physics, University of Rijeka, Division of Experimental and Applied Physics, Rijeka, Croatia*

ivana.gobin@uniri.hr

Introduction. *Candidozyma auris* is an emerging multidrug-resistant fungal pathogen associated with healthcare-associated outbreaks and prolonged persistence on environmental surfaces. Biofilm formation further enhances its tolerance to conventional disinfectants, creating an urgent need for effective decontamination strategies. Ultraviolet C (UVC) irradiation represents a promising no-touch disinfection technology. This study evaluated the efficacy of the Steripro UVC device against 24-hour *C. auris* biofilms formed on different abiotic surfaces.

Materials and Methods. Twenty-four-hour *C. auris* biofilms were developed on plastic, stainless steel, and ceramic surfaces. Biofilms were exposed to Steripro UVC irradiation at distances of 60 and 120 cm for 30 or 60 minutes. Surviving cells were quantified by colony counting and expressed as $\log_{10}$ CFU/mL. Log and percentage reductions relative to untreated controls were calculated. In addition, scanning electron microscopy (SEM) was performed to evaluate UVC-induced structural alterations of the biofilms.

Results. The efficacy of Steripro UVC irradiation depended on both surface type and exposure conditions. Complete eradication of biofilms formed on stainless steel was achieved under all tested conditions, corresponding to a 100% reduction in viable cells. Plastic surfaces showed the highest susceptibility following 60 minutes of irradiation at 60 cm, achieving an 85.8% reduction and a 4.59-log decrease in viable biofilm cells. Ceramic surfaces demonstrated the greatest reduction after 30 minutes of exposure at 60 cm (90.3%), whereas increased irradiation distance reduced treatment efficacy. SEM analysis supported the microbiological findings by revealing disruption of the biofilm architecture and extensive cellular damage following UVC treatment.

Conclusions. Steripro UVC irradiation demonstrated potent activity against 24-hour *Candidozyma auris* biofilms, although treatment efficacy was strongly influenced by surface characteristics and irradiation parameters. Complete elimination of biofilms on stainless steel, substantial reductions on plastic and ceramic surfaces, and SEM-confirmed structural disruption of the biofilm support

the potential application of Steripro UVC technology as an adjunct environmental decontamination strategy for preventing the persistence and transmission of *C. auris* in healthcare settings.

Keywords: *Candidozyma auris*, UVC irradiation, Steripro, biofilm, scanning electron microscopy

P14

Antimicrobial Potential of Plasma-Activated Water on Selected Microorganisms

Jasna Mrvčić, Karla Hanousek Čiča, Damir Stanzer, Višnja Stulić, Ivan Karlo Cingesar, Tomislava Vukušić Pavičić

Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

jmrvcic@pbf.unizg.hr

Non-thermal plasma is a promising food processing technique with various applications and advantages. In recent years, plasma-activated water (PAW) has attracted increasing scientific attention as an innovative antimicrobial and disinfection agent. PAW is produced by treating water with non-thermal plasma discharges. During this process, various reactive oxygen and nitrogen species, such as hydrogen peroxide, nitrate, nitrite, and transient radicals, are generated. Due to the presence of these reactive compounds, PAW exhibits pronounced oxidative and antimicrobial properties.

The aim of this study was to examine the antimicrobial potential of PAW on selected Gram-positive and Gram-negative bacteria and yeast after 3-hour and 24-hour exposure. PAW was generated using a gas-phase plasma reactor with a treatment time of 7 minutes and applied voltages of 20 kV and 30 kV. The working gases were argon, oxygen, and nitrogen. Furthermore, the physicochemical characteristics of the generated PAW were determined, including pH, electrical conductivity, temperature, and concentrations of hydrogen peroxide, nitrates, and nitrites.

The results revealed substantial reductions in microbial populations, demonstrating the antimicrobial effectiveness of plasma-activated water. Moreover, the efficiency of microbial inactivation depended considerably on the plasma process conditions and the type of working gas used during treatment. These findings support the potential application of PAW as a sustainable, eco-friendly alternative to traditional chemical-based disinfection methods.

This work was supported by the Croatian Science Foundation under the project number HRZZ-IP-2024-05-9863.

P15

What About Interaction of Carbon Nanotubes and *Fusarium* spp. Fungi?

Sunčana Včelik[1], Ivana Borišev[2], Aleksandar Đorđević[2], Danica Jović[2], Biljana Crevar[1], Marina Barišić[1], Tihomir Kovač[1]

[1]*Department of Applied Chemistry and Ecology, Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Croatia*
[2]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia*

tihomir.kovac@ptfos.hr

Carbon nanotubes (CNTs), including single-walled and multi-walled variants, are increasingly investigated for their environmental and biological interactions, particularly regarding filamentous fungi of the genus *Fusarium*. This review summarizes current knowledge on the effects of CNTs on *Fusarium* spp. fungi growth, cellular integrity and metabolic activity, with emphasis on underlying toxicological mechanisms. Evidence from recent studies indicates that CNT exposure can significantly inhibit mycelial growth and spore germination in species such as *Fusarium oxysporum* and *Fusarium graminearum*. The magnitude of these effects is strongly dependent on CNT physicochemical properties, particularly surface functionalization, dispersion stability and concentration. Mechanistically, CNTs exert antifungal activity primarily through physical interactions with fungal cell structures and the induction of oxidative stress. Direct contact between CNTs and fungal hyphae has been associated with membrane disruption, cell wall deformation and impaired nutrient transport. Concurrently, exposure to CNTs leads to elevated intracellular reactive oxygen species (ROS) levels, resulting in oxidative damage to lipids, proteins and nucleic acids. This oxidative imbalance subsequently affects key metabolic pathways, including antioxidant defence systems and secondary metabolism. Emerging evidence also suggests potential modulation of mycotoxin biosynthesis pathways in *Fusarium* spp. fungi, although responses appear species- and condition-dependent. In addition, some studies indicate limited fungal-mediated transformation or surface modification of CNTs via extracellular oxidative enzymes, although complete biodegradation has not been demonstrated. Overall, the interaction between CNTs and *Fusarium* spp. fungi is complex and governed by both nanomaterial properties and fungal physiological responses. Understanding these interactions is essential for evaluating the environmental risk of CNTs and their potential application as antifungal agents in agricultural and biotechnological systems.

This study was supported by the project Studies of Bioactive Food Components with New Possibilities of Use for Healthy Nutrition and Nutritional Supplements (BioActFood, NPOO 581-UNIOS-93), funded by the European Union -NextGenerationEU through the Recovery and Resilience Mechanism.

P16

Probiotics or Pathogens? Awareness of *Bacillus* spp. In the Context of Food and Healthcare

Sabina Fijan[1], Nastja Lorber[2], Barbara Kegl[1]

[1]*University of Maribor, Faculty of Health Sciences, Maribor, Slovenia*
[2]*Healthcare Centre Ptuj, Ptuj, Slovenia*

sabina.fijan@um.si

Members of the genus *Bacillus* are ubiquitous, spore-forming microorganisms widely distributed in soil, water, food, and the gastrointestinal tract of humans and animals. Due to their resistance and metabolic versatility, they play a dual role in food and clinical microbiology, encompassing both beneficial and pathogenic representatives. Recent phylogenomic advances have led to taxonomic reclassification of the former Bacillus genus into several new genera, including *Bacillus sensu stricto*, *Alkalihalobacillus*, *Brevibacillus*, *Priestia*, and *Heyndrickxia*. While these changes better reflect evolutionary relationships, they complicate scientific communication and applied understanding, particularly in food safety and probiotics.

Certain species with probiotic strains, such as *Heyndrickxia coagulans*, *Priestia megaterium*, and *Alkalihalobacillus clausii*, are used in foods, supplements, and clinical practice to support gastrointestinal health and immune function. In contrast, species such as *Bacillus cereus* are well-known causes of foodborne intoxications and opportunistic infections. This highlights the importance of strain-level identification and safety evaluation.

This study aimed to assess healthcare workers' knowledge of *Bacillus* spp. as probiotics and pathogens. A cross-sectional study was conducted among 50 healthcare workers at a primary healthcare center using a structured questionnaire covering microbiological knowledge, probiotics, pathogenic species, and awareness of probiotic strains. Data were analyzed descriptively.

Results showed that although most participants had heard of *Bacillus* spp., they mainly associated them with pathogenicity and food risks. General knowledge of probiotics was good; however, awareness of spore-forming probiotic *Bacillus* strains, their safety, and transient gut presence was limited. Knowledge of recent taxonomic changes was also low.

These findings indicate a significant knowledge gap among healthcare professionals. Targeted education on the dual role of *Bacillus* spp., strain-specific safety, and taxonomic updates is needed to support accurate communication in food microbiology and probiotic practice.

P17

Kimchi as a Functional Fermented Food: Evidence-Based Effects on Metabolic Health and Gut Microbiota

Sabina Fijan[1], Klara Žižek[2], Jasmina Golenko[2], Maja Strauss[1]

[1]*University of Maribor, Faculty of Health Sciences, Maribor, Slovenia;*
[2]*University Medical Centre Maribor, Maribor, Slovenia*

sabina.fijan@um.si

Fermented foods represent an important component of traditional diets and are increasingly recognized for their role in human health. Kimchi, a traditional Korean fermented vegetable product, is rich in lactic acid bacteria (LAB), including *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides*, and *Weissella koreensis*, as well as vitamins, minerals, dietary fibre, and bioactive compounds. This study aimed to evaluate the impact of kimchi consumption on human health through a structured review of recent scientific evidence.

A systematic literature search was conducted in PubMed, ScienceDirect, CINAHL, and BioMed Central databases using predefined keywords and Boolean operators. The selection process followed PRISMA guidelines. Out of 273 identified records, six studies met the inclusion criteria and were analysed.

The findings suggest that kimchi consumption is linked to multiple health benefits. These include modulation of the gut microbiota, an increase in the abundance of beneficial bacteria (e.g., *Bifidobacterium* spp.), and suppression of potentially harmful microorganisms. Clinical and observational studies report reductions in body fat, serum triglycerides, total and LDL cholesterol, and blood pressure, alongside improvements in glucose metabolism and insulin sensitivity. Additionally, kimchi intake has been linked to reduced inflammatory markers, improved symptoms of irritable bowel syndrome, and lower prevalence of atopic dermatitis.

Despite these benefits, kimchi contains relatively high levels of sodium, which may pose a risk for individuals with hypertension or kidney disease. However, its potassium content and bioactive compounds may partially counterbalance these effects.

In conclusion, kimchi represents a promising functional food with significant potential to support metabolic health and gut homeostasis. Moderate consumption may contribute to disease prevention, although further well-designed clinical trials are needed to establish optimal intake and long-term effects.

P18

From Formulation to Functionality: Comparative Insights into Postbiotic Preparation and Bioactivity

Martina Banić, Kristina Dučkić, Jasna Novak, Andreja Leboš Pavunc, Katarina Butorac, Jagoda Šušković, Blaženka Kos

Laboratory for Antibiotic, Enzyme, Probiotic and Starter Cultures Technology, University of Zagreb Faculty of Food Technology and Biotechnology, Zagreb, Croatia

jasna.novak@pbf.unizg.hr

Postbiotics are preparations of inanimate microorganisms and/or their components that confer various health benefits on the host. This study aimed to compare different methods for preparing postbiotics from lactic acid bacteria (LAB) previously isolated from donkey milk, breast milk and sauerkraut brine and thoroughly characterised. Based on our earlier research, thermal treatment at 80 °C for 15 min was selected as the optimal method for LAB cells inactivation. Several probiotic preparations were prepared, including cell-free supernatant (CFS), heated cells (HC), complete heat-treated culture (CHTC), and a combination of HC and CFS. In addition, functional properties of live LAB cells (LC) and live cell culture (LCC) were evaluated to compare the bioactivity of live and inanimate preparations. CFSs were prepared by centrifugation followed by filtration, which effectively removed bacterial cells, while thermal treatment used for the preparation of HC and CHTC samples successfully inactivated bacterial cells. Antimicrobial activity was strongest in CFS, LCC, and CHTC samples, although it varied with both the bacterial strain and the preparation method. Thermal treatment did not significantly affect the antimicrobial activity of complete cell cultures in any LAB strain except *Lactiplantibacillus plantarum* DM1. However, thermal treatment significantly reduced proteolytic activity in complete cell cultures of all strains. Postbiotic preparations exhibited strong antioxidant potential, as determined by DPPH and ABTS radical-scavenging assays. Overall, these findings highlight the influence of preparation methods and strain-specific characteristics on postbiotic bioactivity and provide valuable insights for optimising postbiotic production for various applications.

P19

Biopreservative Potential of Rosemary Water Extract in Extending the Shelf Life of Sardine (*Sardina pilchardus*) and Gilthead Sea Bream (*Sparus aurata*)

Iva Čanak, Tea Sokač-Cvetnić, Tibor Janči, Ksenija Markov, Mia Kurek

University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia

iva.canak@pbf.unizg.hr

The preservation of fresh fish remains a major challenge due to rapid microbial proliferation and biochemical changes that negatively affect product quality and shorten shelf life. Growing consumer demand for natural food preservatives has encouraged the exploration of plant-based compounds as alternatives to conventional synthetic additives. Rosemary (*Rosmarinus officinalis* L.) has attracted particular attention because of its well-documented antimicrobial and antioxidant properties.

The objective of this study was to investigate the potential of rosemary water extract, used at different concentrations and in combination with chitosan, to improve the microbial stability and extend the shelf life of whole sardines (*Sardina pilchardus*) and gilthead seabream (*Sparus aurata*) fillets during refrigerated storage. The effectiveness of the treatments was evaluated through microbiological monitoring carried out throughout the storage period.

The application of rosemary extract resulted in a noticeable reduction in microbial growth compared with untreated control samples. Furthermore, combining the extract with chitosan enhanced its preservative action, contributing to a greater delay in spoilage processes. The effectiveness of the treatment depended on the concentration of rosemary extract applied, with treated fish maintaining acceptable microbiological quality for a longer period than the controls.

These results highlight the potential of rosemary water extract as a natural preservation agent for seafood products. Its use, especially in conjunction with chitosan, proved effective in prolonging the shelf life of both whole sardines and gilthead seabream fillets stored under refrigeration, offering a sustainable approach to maintaining seafood quality and safety.

P20

Potential of Fungal and Algal Extracts for the Development of Topical Formulations

Nina Čuljak[1], Ivana Repić[1], Iva Čanak[1], Mojca Čakić-Semenčić[2], Ines Kovačić[3], Gioconda Millotti[3], Ksenija Markov[1], Jadranka Frece[1]

[1]*Laboratory for General Microbiology and Food Microbiology, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*
[2]*Laboratory for Physical Chemistry and Corrosion, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*
[3]*Faculty for Natural Sciences, Juraj Dobrila University of Pula, Pula, Croatia*

jadranka.frece@pbf.unizg.hr

The growing demand for natural bioactive compounds has increased interest in fungi and algae as sustainable sources of antimicrobial and antioxidant agents for topical applications. Marine macroalgae and fungal species represent promising sources of biologically active molecules with potential applications in skin care and health.

In this study, aqueous and ethanolic extracts of the macroalgae *Caulerpa cylindracea*, *Dictyota dichotoma*, *Padina pavonica*, *Halimeda tuna*, *Halopteris scoparia*, and *Laurencia obtusa*, as well as extracts of shiitake (*Lentinula edodes*) and reishi (*Ganoderma lucidum*) mushrooms, were evaluated for their bioactive potential. Macroalgae extracts were prepared from dried and powdered biomass by maceration (1:10 w/v) in water or 70% ethanol for 24 h at room temperature, followed by centrifugation. Fungal extracts were prepared using Microwave-Assisted Extraction and Ultrasound-Assisted Extraction under different extraction conditions. Antimicrobial activity was assessed against selected skin-associated pathogenic microorganisms, including *Staphylococcus aureus*, *Cutibacterium acnes*, *Candida albicans*, *Streptococcus pyogenes*, and *Staphylococcus epidermidis*. Protein profiles were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), while antioxidant potential was determined through glutathione (GSH) quantification.

The extracts exhibited diverse bioactive properties, with observed differences in antimicrobial efficacy and antioxidant capacity depending on species, solvent, and extraction method. SDS-PAGE analysis revealed distinct protein patterns among algal and fungal extracts, indicating variability in their biochemical composition. Several extracts showed inhibitory activity against skin pathogens, suggesting the presence of antimicrobial compounds. Antioxidant analysis demonstrated variability among samples, with the ethanolic extract of *Dictyota dichotoma* showing the highest glutathione concentration.

Overall, the results support the potential of fungal and algal extracts as natural sources of bioactive compounds for topical applications. Their combined antimicrobial and antioxidant properties highlight their promise for the development of innovative and sustainable formulations for skin health. Further studies are required to identify active compounds and evaluate their stability and efficacy in formulated products.

P21

Beyond *Fusarium*: The Impact of Non-*Fusarium* Moulds on *Fusarium* Mycotoxin Contamination in Wheat

Manuela Zadravec, Mislav Unger, Vesna Jaki Tkalec, Sanja Furmeg, Irena Perković, Hrvoje Krajina, Mirta Vukičević, Tina Lešić, Ana Vulić

Croatian Veterinary Institute, Zagreb, Croatia

zadravec@veinst.hr

Wheat (*Triticum* spp.) represents one of the most extensively cultivated cereal grains globally and plays a crucial role in both human and animal nutrition. However, wheat commodities are frequently contaminated by fungal pathogens and their associated mycotoxins, posing significant threats to food safety and quality. Among the mycotoxigenic fungi, *Fusarium* species and their secondary metabolites are predominantly detected in wheat samples. Mycotoxins are toxic fungal metabolites synthesised under suboptimal environmental conditions that inhibit normal fungal development. Beyond abiotic environmental factors, fungal growth dynamics are additionally influenced by antagonistic microorganisms, including competing fungi from diverse genera. This study examined 29 wheat samples collected immediately post-harvest from agricultural Croatian fields during the 2024 growing season. Fungal identification was conducted through morphological characterisation coupled with species-specific gene sequencing analysis. Mycotoxin extraction was performed using an acidified acetonitrile/water solution (80:20, v/v), with subsequent quantitative analysis conducted via liquid chromatography-tandem mass spectrometry (LC-MS/MS). Statistical analyses were employed to determine correlations between fungal presence and mycotoxin occurrence patterns. The most robust and consistent statistical associations were observed for non-*Fusarium* moulds such as *Cladosporium tenuissimum*. The presence of this species demonstrated significant positive correlations with T-2 and HT-2 toxins while exhibiting borderline statistical significance for 15-acetyl-deoxynivalenol. These findings suggest that *C. tenuissimum* may function as an indicator for environmental conditions conducive to elevated trichothecene contamination. Additional significant associations were identified between *Alternaria infectoria* presence and HT-2 toxin, as well as between *Penicillium manginii* occurrence and deoxynivalenol. Conversely, no statistically significant correlations were established between other analysed mycotoxins, including both parent compounds and their metabolites, and any of the fungal species examined. These results demonstrate that relationships between fungal diversity and mycotoxin contamination are characterised by selective, nonlinear associations that are predominantly determined by localised agroecological conditions and environmental variables.

P22

Impact of Chestnut Blight Fungus (*Cryphonectria parasitica*) on the Diversity and Structure of Chestnut Endophytic Fungal Communities

Dino Davosir[1], Lucija Nuskern[1], Ana Diaz Zuniga[2], Antonio Vidaković[3], Marilena Idžojtić[3], Igor Poljak[3], Mirna Ćurković-Perica[1], Iva Franić[2], Simone Prospero[2], Marin Ježić[1]

[1]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*
[2]*Institute of Forest Genetics, Dendrology and Botany, Faculty of Forestry and Wood Technology, University of Zagreb, Zagreb, Croatia*
[3]*Swiss Federal Research Institute WSL, Birmensdorf, Switzerland*

dino.davosir@biol.pmf.hr

Invasive fungal pathogens significantly influence the vitality of forest ecosystems. A prominent example is the phytopathogenic ascomycete *Cryphonectria parasitica*, the causative agent of chestnut blight disease, which severely affects chestnut populations by producing often-lethal cankers on the bark. Beyond damaging the trees, pathogenic fungi are known to destabilise core endophytic fungal communities, thus indirectly influencing host physiology. To investigate the impact of *C. parasitica* infection on the community structure of other cultivable endophytic fungi, a sampling campaign was undertaken in three chestnut populations in continental Croatia. Cultivable fungi were isolated from cankers of varying disease stages: active, intermediate, and passive (healing) cankers, as well as from healthy areas of infected trees, and from trees without cankers. Isolated endophytes were identified by amplification and sequencing of the ITS genomic region. A total of 628 individual isolates were obtained, belonging to 63 fungal species. Apart from *C. parasitica*, which dominated the fungal communities in infected trees, the most common taxa included *Pezicula pseudocinnamomea*, *Biscogniauxia nummularia*, *B. mediterranea*, *Sirococcus smithogilvyi* and *Coryneum modonium*, some of which are known phytopathogens. Alpha diversity indices revealed increased dominance and lower evenness in the endophyte population structure of cankers compared with healthy areas of infected trees or uninfected trees. Endophyte communities from active and intermediate cankers differed significantly more from those originating from canker-less chestnut trees, compared to those from passive cankers, signifying the normalisation of endophytic mycobiota upon canker healing. Building on alpha diversity analyses, beta diversity analyses revealed that the chestnut population of origin was also an important determinant of endophyte community structure. Additional metagenomic analyses of the collected samples will give us a deeper insight into the effects of C. parasitica infection on the total fungal communities of chestnut.

P23

Rising Stolbur Incidence in Croatia: From Vineyards to Field Crops

Ivana Križanac[1], Tihana Vidaković Jelačić[1], Zrinka Drmić[1], Dario Ivić[1], Maja Pintar[1]

[1]*Croatian Agency for Agriculture and Food, Centre for Plant Protection, Croatia*

ivana.krizanac@hapih.hr

Stolbur is a disease of potato caused by the phytoplasma *'Candidatus Phytoplasma solani'*, transmitted by insect vectors, most commonly planthoppers of the Auchenorrhyncha group. The disease is widespread across Europe, and its occurrence in potato crops has increased in recent years, resulting in significant reductions in both yield quantity and quality. Typical symptoms include leaf discoloration (yellowing to purpling), leaf curling, formation of aerial tubers, and the development of deformed, rubbery tubers. Infected tubers are more susceptible to storage diseases and deteriorate rapidly, which complicates industrial processing. Tuber samples were collected from multiple locations in Bjelovar-Bilogora and Osijek-Baranja counties, and laboratory analyses confirmed the presence of *'Ca. P. solani'* in all samples. Consequently, infected tubers are unsuitable for industrial processing. It is estimated that at least 2,500 tons of potatoes were affected, resulting in significant economic losses. Long-term monitoring of phytoplasma vectors in vineyards has shown that species of the genus *Reptalus* and *Hyalestes obsoletus*, both confirmed carriers of *'Ca. P. solani'*, are present and widespread in Croatia. Given the first economically significant occurrence of stolbur on potato in Croatia, as well as the mass occurrence of rubber disease of sugar beet in 2023, further research is needed to identify potential vectors in arable crops and to develop effective strategies for preventing or reducing the spread of this disease.

P24

The Impact of *'Candidatus Phytoplasma solani'* Effector Proteins SAP11-like and SAP54-like on the Expression of Genes Encoding Antioxidative Enzymes in the *Arabidopsis thaliana* Model

Iva Marković, Dino Davosir, Stella Patajac, Martina Šeruga Musić

Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

iva.markovic@biol.pmf.hr

Phytoplasmas (genus *'Candidatus Phytoplasma'*) are insect-transmitted uncultivable bacteria infecting a wide range of plant species. As endocellular pathogens, they secrete effectors directly into the host cell, utilizing them to modulate developmental and metabolic processes as well as the host immune responses. In infected plants, oxidative stress conditions often occur, with an increase in the production of reactive oxygen species (ROS). In order to ensure protection from ROS, plants developed a complex antioxidative system, comprising enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), etc. Within this study, we aimed to examine the impact of *'Ca. P. solani'* SAP11-like and SAP54-like effector proteins on the gene expression of enzymes involved in oxidative stress response in the *Arabidopsis thaliana* model. In our previous research, we obtained transgenic arabidopsis lines overexpressing the SAP11-like and the SAP54-like genes. Tissue of 15-day-old wild-type and transgenic seedlings was used for isolation of total RNA using the commercial kit NucleoSpin RNA Plant (Macherey-Nagel, Düren, Germany). Subsequently, cDNA was synthesized by reverse transcription, and the expression of genes *AtSOD*, *AtGSTU24*, *AtCAT1*, *AtCAT3*, *AtGPX1*, *AtGPX4*, *AtGPX7* and *AtGPX8* was examined by qPCR and determined using the comparative ($\Delta\Delta C_t$) method. The variations in gene expression patterns between effectors and different lines were observed. Among all examined genes, *AtGSTU24* had the highest upregulation in all transformed lines, while the expression of *AtSOD* was not significantly different in the transformed lines. Both catalase genes, *AtCAT1* and *AtCAT3*, showed a trend of slight downregulation. For glutathione peroxidase isoforms analyzed, a general trend of downregulation was observed in the SAP54-transformed lines for *AtGPX1* gene expression, while the SAP11-transformed lines showed a more significant downregulation for *AtGPX4* and *AtGPX7*. Moreover, a significant correlation between *AtGPX4* and *AtGPX7* expression levels was found, pointing to similar regulatory mechanisms during plant-phytoplasma interactions. To the best of our knowledge, this is the first study on the impact of *'Ca. P. solani'* effectors on the antioxidative enzyme gene expression, providing preliminary insights into the role of phytoplasma effectors in oxidative stress and activation of the antioxidative system in affected plants.

P25

Persistence and Infectivity of Stable Plant Viruses in Growing Substrates

Irena Bajde[1,2], Jakob Brodarič[1], Ana Vučurović[1], Nataša Mehle[1,3].

[1]*National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana, Slovenia*
[2]*Jožef Stefan International Postgraduate School, Ljubljana, Slovenia*
[3]*University of Nova Gorica, School for Viticulture and Enology, Vipava, Slovenia*

irena.bajde@nib.si

Stable plant viruses can persist in the environment for extended periods, representing an important epidemiological risk in intensive horticultural production systems. In our previous research, we demonstrated that infectious particles of several stable plant viruses can persist in water for months under favourable conditions while retaining infectivity. However, considerably less is known about virus persistence in solid organic matrices, such as growing substrates.

Growing substrates may act as environmental reservoirs, since small amounts of plant debris often remain after plant removal and can harbour infectious virus particles. Here, we investigate virus persistence and the ability of contaminated substrates to cause infection over time after plant removal, using tomato as a model system and focusing on tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), and tomato bushy stunt virus (TBSV).

Experiments are conducted under controlled greenhouse conditions by introducing healthy tomato seedlings or seeds into previously contaminated substrates. The study addresses contamination scenarios involving multiple viruses, reflecting conditions encountered in agricultural production systems. Virus presence in substrates and transmission to test plants are assessed by reverse transcription quantitative PCR following exposure experiments. Ongoing work extends to additional virus combinations, including potato virus Y (PVY) and tomato apical stunt viroid (TASVd), and explores virus persistence in composted plant material to evaluate the potential role of organic waste streams in virus survival and reintroduction into production systems.

These findings provide new insights into the epidemiological role of growing substrates as sources of infection with stable plant viruses and support improved risk assessment and management strategies for the safe reuse of plant production materials.

P26

Insights Into Genetic Population Structure and Diversity of Cucumber Mosaic Virus in Serbia

Katarina Zečević[1], Ana Vučurović[2], Dušica Kovačević[1], Ivana Stanković[1]

[1]*Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;*
[2]*National Institute of Biology, Ljubljana, Slovenia*

katarina.zecevic@agrif.bg.ac.rs

Cucumber mosaic virus (CMV; species *Cucumovirus CMV*, genus *Cucumovirus*, family *Bromoviridae*) is recognized as one of the viruses with a destructive impact on the production of various vegetable, industrial, and ornamental plants worldwide. In Serbia, CMV is likewise considered among the most economically important plant viruses.

Numerous surveys conducted since 2006 in crops such as tomato, pepper, tobacco, and cucurbits have consistently demonstrated its presence at varying frequencies, both in single and mixed infections with other viruses. CMV has occasionally posed a threat to ornamental plant production, including tulip, impatiens, and garden peony. In addition, it has been detected in the succulent Tuisan peperomia and in the perennial Chinese wisteria, often found in urban green areas and private gardens. Further analyses have revealed the presence of CMV satellite RNAs (satRNAs), including both necrogenic and non-necrogenic variants, particularly in tomato.

Beyond survey-based studies, population analyses have shown substantial genetic variability within the CMV population in Serbia, including multiple genetic variants. Phylogenetic analyses of all five ORFs indicate that all three CMV subgroups exist, with subgroup IA being predominant. Evidence of genetic exchange through recombination and reassortment highlights the dynamic nature of the CMV population. Recombination events between subgroups IA and II have been confirmed in both RNA 2 and RNA 3 segments, while reassortment events, although less frequent, include both IA/IB and IA/II combinations. Notably, a variant exhibiting both reassortment and recombination has also been identified.

Extensive surveys and molecular analyses conducted over the past two decades highlight not only the widespread distribution but also the remarkable genetic diversity of CMV populations circulating in Serbia. The coexistence of multiple genetic variants, including recombinant and reassortant isolates, as well as diverse satRNA types, reflects the complexity of CMV molecular ecology and its adaptive potential across hosts and environments. These findings emphasize the necessity of continuous surveillance and in-depth molecular characterization to

better understand CMV population structure and evolutionary dynamics, ultimately supporting the development of effective management strategies to mitigate its impact on agricultural and horticultural production in Serbia.

P27

HTS and AI-Powered Analysis of Viromes in Hops and the Environment

Tanja Guček[1], Sebastjan Radišek[1], Alenka Guček[2], Jernej Jakše[3]

[1]*Slovenian Institute for Hop Research and Brewing, Žalec, Slovenia*
[2]*Jožef Stefan Institute, Artificial Intelligence Laboratory, Ljubljana, Slovenia*
[3]*University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia*

tanja.gucek@ihps.si

Plant viruses and viroids represent a major threat to global food security, causing substantial annual crop losses worldwide and severe economic damage. Since no effective pesticides are available against these pathogens, rapid, reliable, and comprehensive diagnostics are essential for disease prevention and sustainable crop production. Conventional targeted diagnostic methods, including ELISA, PCR, and Sanger sequencing, depend on prior knowledge of the pathogen and are therefore limited in detecting novel or highly divergent viral agents.

The project "EnviroViroSeq: Advanced HTS-based analysis of viromes in hops and the environment supported by artificial intelligence" (J4-70612; ARIS) addresses these challenges through the implementation of high-throughput sequencing (HTS), with particular emphasis on Oxford Nanopore sequencing technology. The project focuses on comprehensive characterization of viromes in hops and associated environmental samples from selected Slovenian agroecosystems. Hops were chosen as a model economically important crop, while surrounding vegetation and irrigation water are included to investigate potential environmental reservoirs and transmission pathways.

A central objective of the project is the development of AI-supported bioinformatic pipelines that will optimize HTS virome analysis workflows, improve pathogen detection accuracy, and accelerate identification of both known and novel viruses and viroids. Artificial intelligence approaches will be integrated into data processing, taxonomic classification, comparative virome analyses, and prediction of optimal sequencing and enrichment strategies for different sample types. The project also combines Nanopore sequencing with comparative analyses across other HTS platforms and introduces a novel CRISPR-Cas13-based targeted enrichment method for RNA pathogens.

The generated sequencing datasets will enable phylogenetic analyses, investigation of virus–host–environment interactions, and evaluation of AI-assisted approaches for routine diagnostics. The final goal is the validation of a robust Nanopore-based diagnostic workflow compliant with EPPO standards and suitable for routine application in plant health laboratories. By integrating HTS, CRISPR-based enrichment, and artificial intelligence, the project will contribute

to the development of next-generation diagnostic tools, enhanced biosecurity, and sustainable management of plant viral diseases in agriculture.

P28

Single-Cell Sequencing of Coccolithophore Phycospheres Reveals Specific Interactions with Bacterial Communities

Lucija Berlančić, Frederic Chaux, Jelena Godrijan

Ruđer Bošković Institute, Zagreb, Croatia.

lucija.berlancic@gmail.com

Coccolithophores are an important group of marine phytoplankton with a key role in global biogeochemical cycles, particularly carbon cycling in marine ecosystems. Understanding these interactions is essential for resolving microbially mediated processes that influence ocean ecosystem functioning and environmental change. However, their interactions with associated bacterial communities inhabiting the phycosphere remain insufficiently characterized. This study aims to investigate the taxonomic composition and functional potential of bacterial consortia associated with individual coccolithophore cells using a single-cell sequencing approach.

Seasonal sampling of phytoplankton was conducted at Martinska (Adriatic Sea), followed by isolation of individual coccolithophore cells together with their associated bacteria. Cells were morphologically identified, and a protocol for capturing their phycospheres was developed. DNA from isolated bacterial cells was amplified and subjected to 16S rDNA barcoding, while coccolithophore chloroplast markers were used for host species identification. Long-read sequencing was subsequently performed using the Oxford Nanopore MinION platform.

Preliminary results provide insight into the diversity of bacterial communities associated with individual coccolithophore cells, indicating variability among samples and suggesting potential host-specific associations. To compare phycosphere-associated communities with the broader surrounding bacterial assemblage, larger seawater volumes were analyzed in parallel. Year-round sampling further enabled assessment of the influence of environmental conditions on coccolithophore-bacterial associations.

By integrating single-cell approaches with metagenomics, this work contributes to a better understanding of microbe-coccolithophore interactions that underpin marine ecosystem stability and biogeochemical cycling in a changing environment.

P29

Antimicrobial Resistance in *Vibrio* spp. from Adriatic Aquaculture: A One Health Perspective

Damir Kapetanović[1], Karla Orlić[1], Irena Vardić Smrzlić[1], Lorena Perić[1], Snježana Kazazić[1], Božidar Kurtović[1], Brigita Novosel[2], Tin Klanjšček[1]

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

kada@irb.hr

Antimicrobial resistance (AMR) in marine bacteria is an increasing concern within the One Health framework, particularly in aquaculture environments where antimicrobial use may contribute to the selection and spread of resistant microorganisms. This study investigated the occurrence and antimicrobial resistance profiles of *Vibrio* spp. isolated from marine fish farms across the Adriatic Sea. A total of 256 *Vibrio* isolates were obtained from seawater, sediments, mussels, and fish collected at aquaculture sites in the Northern, Central, and Southern Adriatic. Isolates were identified using MALDI-TOF MS and 16S rRNA gene sequencing, and antimicrobial susceptibility testing was performed using the disk diffusion method against ten clinically relevant antibiotics.

The results showed widespread resistance among *Vibrio* isolates, with the highest prevalence observed for ampicillin (62.8%), followed by erythromycin (29.4%) and sulfamethoxazole/trimethoprim (23.5%). Resistance to ciprofloxacin and chloramphenicol was rare, while all isolates remained susceptible to meropenem. Multidrug resistance was detected in 29.3% of isolates, with significant regional differences between the Northern, Central, and Southern Adriatic ($p < 0.05$). Isolates belonging to the Harveyi clade exhibited significantly higher resistance frequencies and a greater prevalence of multidrug resistance compared to the Splendidus clade. Environmental matrices also influenced resistance patterns, with sediment-derived isolates showing higher resistance levels than those from seawater.

The detected resistance profiles indicate persistent selective pressures within aquaculture environments and surrounding marine ecosystems. Although critically important antimicrobials generally retained efficacy, the occurrence of multidrug-resistant *Vibrio* spp. highlights the potential role of marine aquaculture as a reservoir and dissemination pathway for AMR determinants. These findings underscore the need for continuous surveillance, responsible antimicrobial stewardship, and the implementation of sustainable aquaculture practices aligned with the One Health approach. The study provides important baseline data on AMR distribution in Adriatic aquaculture systems and contributes to a broader understanding of resistance dynamics in marine environments.

P30

A Conceptual Framework for Integrating Viruses into Invasion Ecology

Milan Danilović-Feist, Sandra Hudina

University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia.

milan.danilovic-feist@biol.pmf.hr

Background: Biological invasions are a major driver of biodiversity loss and economic costs, which led to development of numerous hypotheses to explain why some introduced species become successful invaders. Invasive non-native species often carry symbionts and hitchhikers, including pathogens, that may also establish, spread, and impact native ecosystems, but they have rarely been accounted for in invasion theory. Instead they have been addressed in research on host–pathogen interactions which has generated a parallel set of disease ecology hypotheses, many of which overlap conceptually with invasion hypotheses. Recent studies further suggest that viruses may serve as powerful proxies for invasion dynamics. Their small genomes, rapid mutation rates, and short generation times enable them to evolve alongside their hosts, making them high-resolution tracers of invasion pathways and promising model systems for testing invasion hypotheses and integrating disease ecology with invasion theory.
Aim: This study presents the conceptual framework for integrating viruses as model organisms into invasion hypotheses and for integrating disease ecology with invasion theory.
Methods: The hypothesis list will undergo a three-step triage: (A) assessment of well-supported, high-relevance hypotheses; (B) expert-consensus grouping, retaining only hypotheses judged relevant to viral biology as Primary Hypotheses, with redundant or nested hypotheses grouped under them; (C) cross-referencing of Primary Hypotheses groups against host-pathogen and epidemiological literature to integrate similar hypotheses between the two fields. Literature search will combine invasion-hypothesis terminology with crustacean and macroinvertebrate virology terms (e.g., virome, *Nimaviridae*, Decapoda, Astacidae). Extracted data will be charted by host taxon, pathogen family, applicable hypothesis, evidence type, and system (wild versus aquaculture).
Expected outcomes: The review will yield a conceptual framework integrating viruses into invasion theory, highlighting research gaps and future directions for invasion ecology, invasion genetics, and management.

Keywords: Invasion biology, macroinvertebrates, Viral pathogens, Disease ecology, Decapoda

P31

Virome Characterization and Dynamics in Successful Crayfish Invaders (ViroCray)

Sandra Hudina[1], Denis Kutnjak[2], Antonin Kouba[3], Katarina Bačnik[2], Ljudevit Luka Boštjančić[2], Andrea Belamarić[2], Silvija Černi[1], Anna Koubova[3], Ivana Maguire[1], Milan Danilović-Feist[1]

[1]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*
[2]*National Institute of Biology, Ljubljana, Slovenia*
[3]*Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Czech Republic*

sandra.hudina@biol.pmf.hr

Invasive non-native species (INNS) pose major threats to biodiversity, ecosystem functioning, human well-being, and the global economy. While host-associated microbial communities have recently been recognized as important drivers of invasion success, the virome (virus-associated microbiome) of invasive species remains largely unexplored. Viruses are the most abundant biological entities in the environment and may play a crucial yet underestimated role in invasion dynamics.

The ViroCray project addresses this knowledge gap by investigating virome diversity and dynamics in three successful freshwater crayfish invaders in Europe: signal crayfish (*Pacifastacus leniusculus*), spiny-cheek crayfish (*Faxonius limosus*), and marbled crayfish (*Procambarus virginalis*). Using high-throughput metagenomic sequencing, the project characterizes known and novel viral communities across various host organs, populations, and ecological contexts: comparing native versus invasive ranges, introduction histories, and species contact zones. By integrating field observations with controlled experimental studies, we investigate the occurrence and magnitude of virome homogenization during interspecific contact, while evaluating the relationship between viral diversity, abundance, and overall host health.

Ultimately, this project will elucidate whether the virome acts as a key driver or modifier of biological invasions and help establish a novel framework for predicting ecological risks related to invasions, tracking wildlife pathogen spillover, and improving management strategies for aquatic species globally.

P32

Joint Effects of Microplastic Exposure and Temperature on the Mycelial Growth of *Aphanomyces astaci*, the Crayfish Plague Pathogen

Karolina Pipinić[1], Klara Milešević[1], Ana Previšić[1], Ana Bielen[2], Sandra Hudina[1]

[1]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*
[2]*Laboratory for Biology and Microbial Genetics, Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*

karolina.pipinic@biol.pmf.hr

Populations of native crayfish have been rapidly declining due to multiple interacting stressors: the introduction of non-indigenous crayfish species and the crayfish plague pathogen (*Aphanomyces astaci* Schikora, 1906), climate change and emerging contaminants, including microplastics. The effects of these stressors on disease occurrence are usually considered only in relation to host susceptibility, whereas their effects on the pathogen itself are understudied. In *A. astaci,* mycelium is the developmental stage that causes disease symptoms by spreading through the crayfish internal organs, while zoospores and cysts spread the pathogen and cause initial infection, respectively. This study aimed to determine the joint effects of increased temperature and microplastic exposure on the mycelial growth of *A. astaci* genotypes A and D. These genotypes were selected due to their opposing temperature sensitivities: A as sensitive to elevated temperatures, and D as more tolerant.

A. astaci cultures were grown on PG1 agar plates at 18 °C and 26 °C and simultaneously exposed to microplastics (MP). Polyethylene was used as a representative freshwater MP pollutant, commonly found in crayfish tissues. MP particles were prepared using a homemade mill with liquid nitrogen and sieved to a size range of 125-250 µm. Two MP concentrations were tested: 10 and 100 MPs mL^{-1} – one and two orders of magnitude greater than the highest environmentally relevant concentration measured, respectively. Laboratory-grade sand particles of the same size range and concentrations were used as negative controls. Mycelial radial growth was measured over 7 days and expressed as growth rate [mm/day].

Results revealed no statistically significant differences in genotype D mycelial growth rates between groups, neither related to temperature nor to MP exposure. This suggests that, at least at the mycelial stage and for this genotype, increased temperature, microplastic pollution and their combination may not substantially alter *A. astaci* viability. These results form a baseline for future experiments on the other two life stages of the pathogen: zoospores and cysts, as well as for

infection trials with crayfish hosts. Altogether, this will help elucidate the effects of microplastic pollution and global warming on the host-pathogen dynamics in the studied system.

This research was financially supported by the Croatian Science Foundation under projects DOK-2025-02 and IPCH 2024-04-6513.

P33

RecA Modulation as a Recurrent Suppressor Route in Recombination-Deficient *Escherichia coli*

Lucija Kumek, Filip Božić, Jelena Repar, Ksenija Zahradka, Davor Zahradka

Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

lkumek@irb.hr

Homologous recombination is essential for bacterial genome maintenance, DNA repair and genetic exchange. In *Escherichia coli*, the central recombinase RecA catalyses homologous pairing and strand exchange, but its loading onto single-stranded DNA normally depends on mediator systems associated with the RecBCD and RecFOR recombination pathways. When both systems are impaired, cells show severe defects in viability, recombination and repair. However, previous work identified an alternative RecBCD/RecFOR-independent pathway, termed RecBFI, active in specific *E. coli* genetic backgrounds, suggesting that bacterial recombination can retain unexpected flexibility under strong genetic constraint.

Here we investigate spontaneous suppressor mutations that arise in RecBCD/RecFOR-deficient *E. coli* mutants and restore growth and recombination-associated phenotypes. Fast-growing suppressor isolates were analysed using genetic linkage tests, targeted *recA* sequencing, whole-genome sequencing and functional assays for transductional recombination, UV survival and double-strand break repair. We identified independent suppressor alleles in *recA*, including recurrent isolation of *recA* A53E, indicating that the RecA recombinase itself is a repeated target of selection in these backgrounds. The characterised alleles differ in the extent to which they restore viability, recombination and DNA damage survival, suggesting that suppression is not simply caused by nonspecific RecA activation.

Our results support a model in which modulation of RecA activity can partially compensate for the loss of canonical RecA-loading systems. This points to RecA as a key adaptive node in bacterial homologous recombination and highlights the capacity of microbial genome-maintenance systems to evolve alternative solutions when major repair pathways are compromised. These findings contribute to a broader understanding of bacterial genome stability, stress adaptation and the genetic plasticity of fundamental DNA repair processes.

P34

Growth Temperature as a Factor of Palindrome Recombinogenicity *in vivo*

Ana Slišković Tomić, Ivan-Krešimir Svetec, Marina Svetec Miklenić

Laboratory for Molecular Genetics and Genetic Engineering, Department of Biochemical Engineering, University of Zagreb Faculty of Food Technology and Biotechnology, Zagreb, Croatia

mmiklenic@pbf.hr

Palindromes in DNA are symmetrical sequences consisting of two inverted repeats. Quasipalindromes consists of repeats that may not be completely identical and/or can have central non-palindromic spacer region. Some palindromic sequences in the play important biological roles, usually as *cis*-acting elements. However, genomes of all organisms have been shown to carry palindromes, without any apparent biological role, that can form secondary structures (hairpins and cruciforms) which in turn cause stalling of replication and double strands breaks initiating genetic recombination. Palindrome recombinogenicity depends *in vivo* on several characteristics, with longer palindromes containing shorter spacers being more recombinogenic. Therefore, such palindromes present fragile sites in the genome, have been linked to a number of syndromes in humans (e.g. Emmanuel syndrome) and recognized as one of the potential drivers of genome instability in cancer cells. Extrusion of a palindrome sequence into a cruciform is thermodynamically unfavorable, and driven *in vivo* by increase in negative supercoiling of DNA. In vitro, several factors have been shown to affect the probability of cruciform formation, one of them being the ambient temperature, as higher temperature facilitates initial DNA denaturation required for cruciform extrusion. To investigate if growth temperature influences cruciform extrusion *in vivo*, and thus the potential of palindromes to instigate genetic recombination, we constructed and tested recombinogenicity of a series of perfect and quasipalindromes at 22 °C, 28 °C and 37 °C, using yeast *Saccharomyces cerevisiae* as a eukaryotic model. Results demonstrated that growth temperature had a significant impact on the rate of intrachromosomal palindrome-initiated genetic recombination. To investigate whether this effect might have been important in the evolutionary perspective, we performed a bioinformatic analysis of bacterial genomes and found that genomes of thermophiles contain fewer and shorter palindromes, while the longest palindromes are found in genomes of psychrophiles. Taken together, these findings suggest that growth temperature is an important determinant of palindrome recombinogenicity *in vivo*, shaping the long-term evolution of microbial genome architecture.

P35

DIY Five Fold Multi-Position Magnetic Stirrer

Goran Palijan

University of Osijek, Department of Biology, Osijek, Croatia

gpalijan@biologija.unios.hr

Determination of soil extracellular enzymes depend on buffer extraction step performed on magnetic stirrer. In our laboratory optimum number of parallel extractions is five. Nevertheless, we do not have five magnetic stirrers in the laboratory so we were forced to collect stirrers from different labs ending with different devices which make difficult to ensure equal extraction conditions between soil samples. In such case, constructing a multi-position magnetic stirrer could provide a compact and cost-effective solution. This poster describes the construction of an affordable five-position magnetic stirrer using off-the-shelf components purchased online. The device consists of an aluminium sheet metal casing, with five separate units each comprising a motor, pulse width modulation controller, tachometer, potentiometer, and direction switch. Each stirring position has its own timer. Therefore, if one stirrer fails, the other four remain operational. After 30 minutes of continuous stirring, the stirrers deviated between 0.02% and 6.15% from the set speeds, with an average deviation of 2.43%. The difference between the respective stirrers was 4.7% at 300 rpm, 5.35% at 600 rpm, and 2.06% at 900 rpm, which is comparable to commercial devices (3%–5%). To test the device under relevant conditions, two soil samples of differing organic matter content were used for extraction of the soil phosphatase enzyme. Two extractions were conducted with the same soil batch on two separate days. Statistical analysis showed no statistically significant difference between extractions, hence the stirrer produced consistent results across different stirring occasions. Also, the size of standard deviation was soil type related i.e. not device related. This was achieved at less than a quarter of the price of comparable commercially available magnetic stirrers. The device enabled multiple samples to be stirred at the same speed or simultaneous testing under different conditions, which improved efficiency and enhanced workflow in everyday laboratory work.

P36

Teaching Microbial Biotechnology for Climate Change Mitigation: Educational Resources Developed Within the BIOSHIELD Project

Dunja Šamec, Barbara Medvedec

Department of Food Technology, University North, Croatia

dsamec@unin.hr

Microorganisms are increasingly recognized as key drivers of sustainable solutions for climate change mitigation, environmental protection, and the development of a circular bioeconomy. Equipping future professionals with knowledge and skills in microbial biotechnology therefore represents an important educational challenge. The Erasmus+ project BIOSHIELD (Novel Biotechnological Solutions in Climate Change Mitigation) addresses this need through the development of innovative educational resources that integrate biotechnology, microbiology, sustainability, and climate resilience. In the presentation will introduce the educational outputs developed within the BIOSHIELD project by an international consortium of higher education institutions and associated partners. The project has produced a comprehensive curriculum, student handbook, teaching materials, case studies, practical exercises, and digital learning resources designed to support modern biotechnology education. Particular emphasis is placed on microbial applications in climate change mitigation, including environmental biotechnology, sustainable production systems, and nature-based solutions.

The developed materials promote interdisciplinary learning and enable students to connect theoretical concepts with real-world challenges. Resources have been designed to support both classroom and independent learning, while encouraging critical thinking, problem-solving, and awareness of the role of biotechnology in achieving sustainability goals.

By making these resources openly accessible, the BIOSHIELD project contributes to strengthening biotechnology education and increasing student engagement with topics at the interface of microbiology and climate action. The presented materials may serve as valuable tools for educators seeking to incorporate contemporary examples of microbial biotechnology into their teaching and to prepare students for future challenges in sustainable development.

LIST OF AUTHORS

A	
Alexandra Nola, Iskra	P3
Andrišić, Miroslav	OP15
Auguštin, Dubravka Havaš	P4
Avberšek, Jana	OP15
B	
Babić, Ivana	OP3, OP9
Bačič, Martina	IL1
Bačinić, Anđela	OP1
Bačnik, Katarina	IL1, OP5, OP17, P31
Badovinac, Ivana Jelovica	P13
Bajde, Irena	P25
Banić, Martina	P18
Barišić, Marina	P15
Bech Hansen, Egon	IL5
Bedenic, Dora	IL2
Begić, Gabrijela	P9
Belamarić, Andrea	OP5, P31
Berlančič, Lucija	P28
Bešlić, Ivana	P3
Bielen, Ana	OP5, OP8, P32
Bleker, Carissa	IL1
Borišev, Ivana	P15
Bošnjak, Zrinka	OP7, P8
Božić, Filip	OP13, P33
Božičević, Ivana	P3
Brodarič, Jakob	P25
Bukvič, Veronika	IL1
Buljević Zdjelarević, Petra	CDM3
Butorac, Katarina	P18

C, Č, Ć	
Čakić-Semenčić, Mojca	P20
Čanak, Iva	P19, P20
Cernava, Tomislav	P4
Černi, Silvija	P31
Chaux, Frederic	OP3, OP16, P28
Čičmak, Ana	P7
Crevar, Biljana	P15
Crudo, Francesco	IL6
Čuljak, Nina	P20
Curk, Tomaž	OP17, CDM1, WS2
Ćurković-Perica, Mirna	OP6, P22
Ćurtović Pučar, Ida	P7, P8
Cuturic, Nensi	OP16
Cvijanovic Peloza, Olga	P9
D, Đ	
Dabelić, Sanja	P7
Danilović-Feist, Milan	P30, P31
Davosir, Dino	IL4, P22, P24
Diaz Zuniga, Ana	P22
Dopuđ, Maja	OP15
Đorđević, Aleksandar	P15
Drašković, Isabela	P2
Drčelić, Marina	IL4
Drmić, Zrinka	P23
Dučkić, Kristina	P18
Duvnjak, Sanja	OP15
E	
Erčulj, Špela	IL1
F	
Ferenčak, Ivana	P1, P3
Ferle, Maja	IL1, OP4

..F	
Fiala, Ivan	OP3
Fijan, Sabina	P16, P17
Franić, Iva	P22
Fránová, Jana	OP10
Frece, Jadranka	P20
Frka Milosavljević, Sanja	OP9
Furdek Turk, Martina	OP1
Furmeg, Sanja	P21
G	
Glažar Ivče, Daniela	P3
Gligora Udovič, Marija	OP2
Gobin, Ivana	P9, P10, P12, P13
Godrijan, Jelena	P28
Golenko, Jasmina	P17
Golparian, Daniel	P3
Grabarić Andonovski, Iva	WS1
Gradišnik, Lidija	P5
Gross-Bošković, Andrea	OP12
Guberović, Iva	CDM2
Guček, Alenka	P27
Guček, Tanja	P27
Gutiérrez-Aguirre, Ion	IL1, OP4
H	
Hajdarović, Marina	IL2
Hanousek Čiča, Karla	P14
Havaš, Dubravka	OP9
Hengl, Brigita	OP12
Hižak, Magdalena	OP9
Hlebić, Lucija	OP15
Hoenigl, Martin	IL3, P8
Horvat, Lucija Lulić	P2

..H	
Hrupec, Lucija	OP16
Hruškar, Željka	P1
Huđ, Anastazija	OP1, OP3
Hudina, Sandra	OP5, P30, P31, P32
Humski, Andrea	OP15
I	
Ida Ćurtović Pučar	OP7
Idžojtić, Marilena	OP6, P22
Ivančić-Baće, Ivana	IL8
Ivanić, Maja	OP9
Ivić, Dario	P23
Ivković, Romana	IL7
J	
Jacobsson, Susanne	P3
Jaki Tkalec, Vesna	P21
Jakše, Jernej	P27
Jakšić, Daniela	OP7, P7, P8
Janči, Tibor	P19
Ježić, Marin	OP6, P22
Jovetić, Danka	P7
Jović, Danica	P15
Jurić, Anita	P1
Jurić, Dragan	P1, P3
Jurina, Dunja	OP2
Juzbašić, Tajana	P3
K	
Kapetanović, Damir	P29
Karleuša Mujkić, Ljerka	P9, P12
Karlo Cingesar, Ivan	P14
Katanić, Zorana	OP6
Katinić, Marta	IL2

..K	
Kazazić, Snježana	P29
Kegl, Barbara	P16
Kindl, Marija	P11
Klanjšček, Tin	P29
Koloniuk, Igor	OP10
Kompes, Gordan	OP15
Koprivčić, Dora	P11
Kos, Blaženka	P18
Košiček, Marko	CDM3
Košpić, Karla	OP7
Kouba, Antonin	OP5, P31
Koubova, Anna	P31
Kovač, Tihomir	P15
Kovačević, Dušica	P26
Kovačić, Ines	P20
Kozarić, Marta	OP7, P7, P8
Krajina, Hrvoje	P21
Kranjc, Luka	OP17
Križanac, Ivana	OP11, P23
Krstin, Ljiljana	OP6
Kulaš, Antonija	OP2
Kumek, Lucija	OP13, P33
Kurek, Mia	P19
Kurtović, Božidar	P29
Kutnjak, Denis	IL1, OP4, OP5, OP17, P31
L	
Lakošeljac, Ivana	P10
Lass-Flörl, Cornelia	OP7, P8
Leboš Pavunc, Andreja	P18
Leigh, Deborah Marie	OP6
Lendić, Barbara	OP12

..L	
Lengar, Živa	IL1
Lešić, Tina	P21
Levstek, Marjetka	IL1
Linšak, Željko	P10
Lisičić, Duje	IL2
Lorber, Nastja	P16
Lovrić, Mario	OP9, P4
Luka Boštjančić, Ljudevit	OP5, P31
M	
Magdić, Ivan	OP9
Maguire, Ivana	P31
Mahnič, Aleksander	P5, P6
Maja Šegvić Klarić	OP7
Majhen, Dragomira	P2
Malmstrom, Carolyn M.	IL1
Mamić, Tomislav	IL8
Maraš, Roko	P12
Marasek, Tomasz	SL1
Maravić-Vlahoviček, Gordana	P11
Marceljak Ilić, Mihaela	IL2
Margaria, Paolo	OP11
Marko, Doris	IL6
Markov, Ksenija	P19, P20
Marković, Iva	IL4, P24
Marković, Lana	IL2
Marković, Marija	P1
Marohnić, Marko	OP4
Martin Hoenigl	OP7
Martinić Cezar, Tea	IL7
Matković, Julijana	IL2
Maver, Uroš	P5

..M	
Medvedec, Barbara	P36
Mehle, Nataša	P25
Michl, Kristina	P4
Mijakovic, Ivan	KL2
Mikolajczak, Andrzej	OP15
Milešević, Klara	P32
Millotti, Gioconda	P20
Mitić, Damjan	IL8
Mladineo, Ivona	OP3
Mlakar, Sabina	P5, P6
Mrvčić, Jasna	P14
Mudri, Patrik	P12
N	
Nemeth Blažić, Tatjana	P3
Novak, Jasna	P18
Novaković, Marta	IL8
Novosel, Brigita	P29
Nuskern, Lucija	OP6, P22
O	
Oberhaensli, Simone	OP8
Obranić, Sonja	P11
Orlić, Karla	P29
P	
Paić, Antonia	IL7
Pajek Arambašič, Neža	OP17
Pakiž, Maja	P6
Palijan, Goran	OP1, OP9, P35
Papić, Bojan	OP15
Patajac, Stella	P24
Pavoković, Dubravko	IL2
Peinović, Lovran	OP15

..P	
Peranić, Karla	OP6
Perić, Lorena	P29
Perković, Irena	P21
Peruč, Dolores	P3, P10
Petek, Marko	OP9
Petrić, Jasenka	OP12
Pintar, Maja	P23
Pipinić, Karolina	P32
Pjevac, Petra	OP2
Pleško, Sanja	OP7, P8
Poberžnik, Živa	P6
Poljak, Ema	P13
Poljak, Igor	OP6, P22
Pompe Novak, Maruša	IL4
Popović, Maja	OP6
Previšić, Ana	P32
Pribanić Matešić, Marina	P12, P13
Přibylová, Jaroslava	OP10
Prospero, Simone	P22
R	
Radišek, Sebastjan	P27
Rajnović, Ivana	OP9
Ranogajec Blažeka, Sabina	IL2
Reil, Irena	OP15
Repac Antić, Davorka	P10
Repar, Jelena	OP13, OP14, P33
Repić, Ivana	P20
Rigling, Daniel	OP6
Runtić, Ana	P2
Rupnik, Maja	P5, P6

S, Š	
Samadan Marković, Lara	P1
Šamec, Dunja	OP9, P36
Šarac, Jelena	OP9, P4
Šarić, Ela	OP8
Schmidt-Posthaus, Heike	OP8
Šegvić Klarić, Maja	P7, P8
Šeruga Musić, Martina	IL2, IL4, OP11, P24
Sičić, Iva	P11
Siroglavić, Marko	OP7, P8
Škorić, Dijana	OP11
Slišković Tomić, Ana	P34
Sokač-Cvetnić, Tea	P19
Šoprek Strugar, Silvija	OP15
Špak, Josef	OP10
Spano, Giuseppe	KL1
Špičić, Silvio	OP15
Stanečić, Željka	OP7
Stanković, Ivana	P26
Stanzer, Damir	P14
Stenger-Kovács, Csilla	OP2
Stojković, Ilijana	P1
Strah, Rebeka	IL4
Strauss, Maja	P17
Stulić, Višnja	P14
Stuparević, Igor	IL7
Šunić, Iva	P4
Šušković, Jagoda	P18
Šuto, Sandra	OP15
Svetec Miklenić, Marina	P34
Svetec, Ivan-Krešimir	P34
Sviben, Mario	P7

..S, Š	
Sviličić Petrić, Ines	OP1, OP2, OP3, OP9
T	
Tabain, Irena	P1
Teparić, Renata	IL7
Tomašković, Dora	OP15
Tominc, Kaja	P5
U	
Ulaga, Juraj	IL2
Unemơ, Magnus	P3
Unger, Mislav	P21
V	
Vajda, Jernej	P5
Vardić Smrzlić, Irena	P29
Včelik, Sunčana	P15
Veselá, Jana	OP10
Vidaković Jelačić, Tihana	P23
Vidaković, Antonio	P22
Vihar, Boštjan	P5
Vipotnik, Zala	P6
Vladimir-Knežević, Sanda	P11
Vogrinec, Lana	IL1, OP4, OP17
Volarević, Nilia	P3
Vrana, Ivna	OP1
Vučurović, Ana	OP4, P25, P26
Vukičević, Mirta	P21
Vuković, Rosemary	OP6
Vukušić Pavičić, Tomislava	P14
Vulić, Ana	P21
Z, Ž	
Zadravec, Manuela	P21
Žagar, Mirta	IL8

..Z, Ž	
Zahradka, Davor	OP13, OP14, P33
Zahradka, Ksenija	OP13, OP14, P33
Zdelar Tuk, Maja	OP15
Zečević, Katarina	P26
Žižek, Klara	P17
Žmak, Ljiljana	P3
Žunar, Bojan	IL7
Žutinić, Petar	OP2