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MINISTRY OF EDUCATION, YOUTH AND SPORTS

2nd INTER MICRO project meeting

LIBLICE 2025

PROGRAM

&

ABSTRACT BOOK

MBU Institute of Microbiology Academy of Sciences of the Czech Republic

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

CHARLES UNIVERSITY

This research has been supported by the Ministry of Education, Youth and Sports of the Czech Republic grant Talking microbes - understanding microbial interactions within One Health framework (CZ.02.01.01/00/22_008/0004597)

Obsah

PROGRAM AT GLANCE

Wednesday 10 September

14:00-15:00	*Arrival, check in, poster installation*
15:00-15:10	**Opening the meeting, announcements, instructions (Jan Jansa)**
15:10-15:20	**Welcome address** by the director of the Institute of Microbiology
15:20 – 16:20	**WP 1 – selected stories I**
16:20-16:45	*Coffee break + posters (everyone´s own choice)*
16:45 – 17:10	**WP 1 – selected stories II**
17:10 – 18:00	**WP 1 – Instrumentation and technologies**
18:00 – 20:00	*Dinner*
20:00 – ….	**Posters** with refreshment

Thursday 11 September

…. – 9:00	**Breakfast**
9:00 – 9:20	**WP 2 – selected stories - Encapsulation**
9:20 – 9:40	**WP 2 – selected stories - Innovative delivery systems**
9:40 – 9:55	**WP 2 – selected stories - Advanced barrier models**
10:00 – 10:30	*Coffee break + posters (everyone´s own choice)*
10:30 – 10:40	**WP 3 – selected stories - Overview**
10:40 – 11:10	**WP 3 – selected stories - Microbial colonization and immunity**
11:10 – 11:45	**WP 3 – selected stories - Microbiome gut-brain axis**
11:45 – 12:00	**WP 3 – selected stories - Mechanisms of microbiome actions**
12:00 – 14:00	*Lunch*
14:00 – 14:20	**WP 4 – selected stories - Microbial processes in the environment**
14:20 – 14:35	**WP 4 – selected stories - Bacteria in fungal hyphosphere**
14:35 – 14:55	**WP 4 – selected stories - Global databases in fungal ecology**
14:55 – 16:00	**Reflecting on project progress – The good, The bad and The ugly (Jan Jansa)**
16:00 - 16:30	*Coffee break + posters (everyone´s own choice)*
16:30 - 20:00	- *Pétanque tournament (guided tour of the castle in case of rain)* - **ISAB closed-door meeting, discussion with project PI**
20:00 – ….	*Reception*
21:00 – 21:50	**Mr. Tumor - theatre performance (Ondrej Lidicky)**

Friday 12 Sept 2025

…. – 9:00	**Breakfast and CHECK-OUT**
9:00 – 10:15	**Selected papers from the project**
10:15 – 10:45	**poster winners, petanque results etc, REMOVING POSTERS**
10:45 – 12:00	**Feedback from ISAB, closing the meeting**
12:00 – 14:00	*Lunch and departure*

PROGRAM

Wednesday 10 Sept 2025	
14:00 – 15:00 **Check in**, put **posters on display**	
15:00 – 15:10 **Opening the meeting**, announcements, instructions (**Jan Jansa**)	
15:10 – 15:20 **Welcome address** by the director of the Institute of Microbiology (**Jiří Hašek**)	
15:20 – 16:20 **Workpackage 1 – selected stories I**	
15:20 – 15:40 **ABCF proteins**	
15:20 – 15:35 Decoding resistance: ABCF proteins tailor bacterial responses to antibiotic type and concentration (**Gabriela Balíková-Novotná**)	FT1
15:35 – 15:40 Exploring the antibiotic-responsive regulatory function of ABCF proteins in antibiotic-producing bacteria (**Mufarrah Mehboob**)	LT1 + PT1
15:40 – 16:00 **(Fungal) siderophores**	
15:40 – 15:55 From INTERMICRO to medical mycology (**Vladimír Havlíček**)	FT2
15:55 – 16:00 A bioinformatic approach for the characterization of microbial siderophores from LC-MS datasets (**Jiří Novák**)	LT2 + PT2
Lipocalin-enterobactin complexes revealed by native MS and molecular modelling (**Summra Ahmed**)	PT3
16:00 – 16:20 **Biotransformations**	
16:00 – 16:15 Tailored glycoconjugates derived from human milk oligosaccharides and their bioactivities (**Pavla Bojarová**)	FT3
16:15 – 16:20 Novel fungal α-l-fucosidase exhibiting transfucosylation activity (**Pavlína Nekvasilová**)	LT3 + PT4
Biosynthesis, biodegradation and determination of the non-protein amino acid β-cyano-L-alanine, an intermediate of cyanide detoxification in plants (**Barbora Křístková**)	PT5
Prenylation and geranylation of flavonoids for improved bioavailability and anti-inflammatory effects (**Alice Pomeislová**)	PT6
16:20 – 16:45 **Coffee break**	
16:45 – 18:00 **Workpackage 1 – selected stories II, Instrumentation and technologies**	
16:45 – 17:10 **Bacterial – host interactions**	
16:45 – 17:00 Calcium-loaded acylated segment controls the membrane penetration capacity of repeats in ToXin (RTX) cytolysins (**Jiří Mašín**)	FT4
17:00 – 17:05 *Kingella kingae* RtxA toxin interacts with cell surface glycans and disrupts epithelial barrier integrity (**Waheed Ur Rahman**)	LT4 + PT7
17:05 – 17:10 KhpA/B form a complex with RNase Y as an RNA chaperone in *Streptococcus pneumoniae* (**Jan Keil**)	LT5 + PT8
Linking c-di-AMP homeostasis and cell division regulation in *Streptococcus pneumoniae* (**Tomáš Beneš**)	PT9
Neurotoxic activity of *Bordetella* dermonecrotic toxin at sub-picomolar concentrations (**Ondřej Staněk**)	PT10
Bordetella pertussis toxins drive the emergence of a unique $CD8^+$ T cell subset in the respiratory tract (**Jana Holubová**)	PT11
Extreme C-terminus of the FhaB prodomain is essential for interaction of *Bordetella pertussis* with nasal ciliated epithelial cells (**Ladislav Bumba**)	PT12

	Deciphering the early innate immune response of nasal mucosa to *Bordetella pertussis* infection (**Ludmila Blechová - Brázdilová**)	PT13
	The mystery of the *Bordetella* type III secretion system effector protein BteA (**Tania Romero Allsop**)	PT14
	Bimodal behaviour of intracellular Salmonella (**Milada Kambová**)	PT15
	Cyclic di-GMP signaling and intracellular adaptation of *Aeromonas veronii* in free-living amoebae (**Jan Blumenstein**)	PT16
17:10 – 18:00 **Instrumentation and technologies**		
17:10 – 17:25	Uncovering the microbial metabolome using TimsTOF MS (**Zdeněk Kameník**)	FT5
17:25 – 17:30	Molecular heterogeneity within staphylococcal brain abscesses - MALDI IMS (**Dominika Luptáková**)	LT6 + PT17
17:30 – 17:45	Deciphering host-derived signals that modulate c-di-GMP in *Bordetella* – fluorescence microscopy (**Denisa Vondrová**)	FT6
17:45 – 18:00	Perspectives in proteomics: Picogram-level proteomics workflows (**Saša Vatić**)	FT7
	Facing the challenges of untargeted metabolomics: Our path to workflow development and data integration (**Tommaso Stefani**)	PT18
	Development of software for LC-MS data processing of oligonucleotides (**Evgeniya Biryukova**)	PT19
	Structural dynamics of the CyaA acylated segment drive membrane invasion: Insights from HDX-MS (**Zuzana Kalaninová**)	PT20
	Quantitative cross-linking MS using data-independent acquisition as a novel tool for study of structural rearrangement of proteins in bacteria (**Michael Karpíšek**)	PT21
18:00 – 20:00 **Dinner and free time**		
20:00 – until late **Poster session** (with refreshments and drinks)		

Thursday 11 Sept 2025		
until 9:00 **Breakfast**		
09:00 – 10:00 **Workpackage 2 – selected stories**		
09:00 – 09:20 **Encapsulation**		
09:00 – 09:15	Advanced encapsulation strategies for various biological entities and biomolecules: From spray drying to microfluidic applications (**Viet Tomáš Nguyen**)	FT8
09:15 – 09:20	Targeting biofilm structure and integrity with free and microparticle-based allicin (**Nina Nováková**)	LT7 + PT22
09:20 – 09:40 **Innovative delivery systems**		
09:20 – 09:40	Overview and (potential) applications in the INTER-MICRO project (**František Štěpánek**)	FT9
	Oil marbles as SMEDDS-based carriers for enhanced delivery of curcumin (**Eva Králová**)	PT23
	Glucan particles as an Innovative oral delivery system for cannabidiol (**Adéla Brejchová**)	PT24
09:40 – 10:00 **Advanced barrier models**		

09:40 – 09:55 A new generation of *in vitro* advanced barrier models for evaluating the effects of drugs and food ingredients (**Jitka Viktorová**)	FT10
09:55 – 10:00 Engineering biosimilar mucus: Integrating composition, rheology and bioprinting for *in vitro* barrier model (**Suada Đukaj**)	LT8 + PT25
10:00 – 10:30 **Coffee break**	
10:30 – 12:00 **Workpackage 3 – selected stories**	
10:30 – 10:40 WP3 Overview: Who, what and with whom (**Martin Schwarzer**)	FT11
10:40 – 11:10 **Microbial colonization and immunity**	
10:40 – 10:55 Segmented filamentous bacteria (SFB)-induced intraepithelial lymhocytes differentiation: The critical molecular and cellular players (**Jan Dobeš**)	FT12
10:55 – 11:10 The mechanisms that lead to the polarization of microbiota-specific T cells into intraepithelial lymphocytes (**Iva Pacáková + Anna Jelínková**)	FT13 + PT26
Western diet disrupts anti-tumor immunity and promotes melanoma progression through myeloid and T cell modulation (**Janaina L. S. Donadio**)	PT27
Oral colistin improves the therapeutic response of tumors to anti-PD1 treatment in an IFN-γ-dependent mechanism (**Miloslav Kverka**)	PT28
Depletion of myeloid cells reduces tumorigenesis driven by a high-protein diet in a colitis-associated cancer model (**Veronika Motúzová**)	PT29
Immune response to microbial transglutaminase after polyinosinic-polycytidylic acid (poly I:C) immunization in a murine model (**Maliha Rizwan**)	PT30
The gut microbiome and diet composition of Papua New Guinea rodents among different altitudes from two mountain ranges (**Halil Mert Solak**)	PT31
Interspecific divergence and the effect of neutral processes on microbiota variation in inbred strains derived from eight mouse species (**Leighton J Thomas**)	PT32
Making SPF mice great again: Symbiotic microbiota restoration in SPF mice through dirty facility exposure (**Jakub Kreisinger**)	PT33
11:10 – 11:45 **Microbiome gut-brain axis**	
11:10 – 11:45 **Microbiome gut-brain axis**	
11:10 – 11:20 Introduction (**Petra Procházková**)	FT14
11:20 – 11:25 Microbiome and metabolic disruption in acute vs. severe and enduring anorexia nervosa (**Petra Procházková**)	LT9
11:25 – 11:30 Microbiota-driven effects on the development of a mouse model of anorexia nervosa (**Radka Roubalová**)	LT10
11:30 – 11:35 Ocrelizumab causes changes in microbiome and modulates the immune response depending on the response to multiple sclerosis treatment (**Štěpán Coufal**)	LT11
11:35 – 11:40 Lysate of *Parabacteroides distasonis* prevents severe forms of experimental autoimmune encephalomyelitis by modulating the priming of T cell response (**Zuzana Jirásková Zákostelská**)	LT12
11:40 – 11:45 mini-discussion of the LT9 through LT12	

11:45 – 12:00 **Mechanisms of microbiome actions**	
11:45 – 12:00 Growth-promoting properties of lactic acid bacteria (**Tereza Novotná**)	FT15
Advancing gut microbiome metabolomics: A derivatization-based approach (**Anna Michl**)	PT34
Butyrate alters hepatic drug metabolism and expression of short-chain fatty acid transporters and receptors in the colon of mice with DSS-induced colitis (**Tomáš Hudcovic**)	PT35
Bifidobacterium longum ssp. *longum* CCM 7952-derived peptidoglycan alleviates allergic inflammation through TLR-dependent signaling in a mouse model of airway allergy (**Dagmar Šrůtková**)	PT36
12:00 – 14:00 **Lunch and free time** (for poster viewing, for instance)	
14:00 – 14:55 **Workpackage 4 - selected stories**	
14:00 – 14:20 **Microbial processes in the environment**	
14:00 – 14:15 Bacterial populations active during lignin depolymerization (**Ana Catalina Lara**)	FT16
14:15 – 14:20 Steroids as potential adjuvants: effect on efflux systems and gene expression in *Staphylococcus aureus* (**Jan Špaček**)	LT13 + PT37
Uncovering a novel acetosyringone catabolic enzyme with co-metabolic activity towards chlorinated pollutants (**Michal Strejček**)	PT38
Genomic characterization of groundwater bacterial phylum UBA9089 (**Roman Skala**)	PT39
Harnessing microbial metabolism: A conjugative plasmid-based strategy to enhance degradation of organic contaminants in soils (**Jáchym Šuman**)	PT40
14:20 – 14:35 **Bacteria in fungal hyphosphere**	
14:20 – 14:35 Towards a relevant experimental model of arbuscular mycorrhizal fungal hyphosphere (**Valeriia Belova**)	FT17
Stable isotope probing to disentangle carbon for nitrogen trading in mycorrhizal hyphosphere (**Petra Bukovská**)	PT41
Disentangling mycorrhiza-nitrification interactions in soil by using synthetic nitrification inhibitors (**Jan Jansa**)	PT42
Short-term biocide stress alters soil microbial communities and affects plant growth, carbon stabilization, and enzymatic activity in soil (**Caroline Krug Vieira**)	PT43
14:35 – 14:55 **Global databases in fungal ecology**	
14:35 – 14:50 Harnessing global databases to understand fungal ecology: lessons from GlobalFungi and GlobalAMFungi (**Petr Kohout**)	FT18
14:50 – 14:55 ALFI: A global database of alien fungal introductions (**Vasilii Shapkin**)	LT14 + PT44
GlobalFungi - the global atlas of fungal biodiversity (**Petr Baldrian**)	PT45
GlobalAMFungi – the global atlas of arbuscular-mycorrhizal fungal biodiversity (**Tomáš Větrovský**)	PT46
14:55 – 16:00 **Reflecting on project progress – The good, The bad and The ugly**	
Work – alone and in collaboration, going beyond the state of the arts	

Advanced techniques – benefits and pitfalls	
Reporting and other outputs, incl publications	
Open access and related issues	
Others	
16:00 – 16:30 **Coffee break**	
16:30 – 20:00 **Socializing (sporting activities, excursion, free time)** (more information will follow), **ISAB** – closed-door meeting, drafting report, discussion with project PI + managers (?)	
20:00 – until late: **Reception (BBQ)**	
21:00 – 21:50 **Mr Tumor theatre**	

Late-coming posters:

Interaction of silymarin flavonolignans with human gut microbiota (**Kateřina Valentová, WP1**)	PT47
confidential (**Martin Krov, WP2**)	PT48
Ex germ-free mouse model with natural microbiomes (**Dagmar Čížková, WP3**)	PT49

Friday 12 Sept 2025	
until 9:00 **Breakfast AND CHECK-OUT (important)**	
09:00 – 10:15 **Selected papers from the project**	
09:00 – 09:15 Brodsky et al (2024) New bacterial aryl sulfotransferases: Effective tools for sulfation of polyphenols, doi 10.1021/acs.jafc.4c06771 (**Barbora Petránková**)	FT19
09:15 – 09:30 Vaishnav et al (2025) Protists are key players in the utilization of protein nitrogen in the arbuscular mycorrhizal hyphosphere, doi 10.1111/nph.70153 (**Anukool Vaishnav**)	FT20
09:30 – 09:45 Zmuda et al (2025) The *Bordetella* effector protein BteA induces host cell death by disruption of calcium homeostasis, doi 10.1128/mbio.01925-24 (**Jana Kamanová**)	FT21
09:45 – 10:00 Horníková et al (2025) Genetic background and microbiome drive susceptibility to epicutaneous sensitization and food allergy in adjuvant-free mouse model, doi 10.3389/fimmu.2024.1509691 (**Dagmar Šrůtková**)	FT22
10:00 – 10:15 Slivenecká et al (2025) The *Actinobacillus pleuropneumoniae* apxIV operon encodes an antibacterial toxin-immunity pair, doi 10.1016/j.micres.2024.128043 (**Ladislav Bumba**)	FT23
10:15 – 10:45 **Announcements** (poster winner, sport tournament results etc), **removing posters**	
10:45 – 12:00 **Feedback from ISAB, recommendations, suggestions, closing the meeting**	
12:00 – 14:00 **Lunch and departure**	

For conciseness, only the presenting author names (without co-authors or affiliations) are shown here without any academic titles

FT – full talk – 12 min speaking time + 3 min discussion (or 15+5, or 10+0, as applicable)

LT – lightning talk (5 min, discussion at the end of a block)

LT + PT – lightning talk (5 min, no discussion) accompanied by a poster

PT – poster presentation only

LIST OF POSTER PRESENTATIONS

PT1	Mufarrah Mehboob	EXPLORING THE ANTIBIOTIC-RESPONSIVE REGULATORY FUNCTION OF ABCF PROTEINS IN ANTIBIOTIC-PRODUCING BACTERIA
PT2	Jiří Novák	A BIOINFORMATIC APPROACH FOR THE CHARACTERIZATION OF MICROBIAL SIDEROPHORES FROM LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY DATASETS
PT3	Summra Ahmed	Lipocalin-enterobactin complexes revealed by native mass spectrometry and molecular modelling
PT4	Pavlína Nekvasilová	NOVEL FUNGAL α-l-FUCOSIDASE EXHIBITING TRANSFUCOSYLATION ACTIVITY
PT5	Barbora Křístková	Biosynthesis, biodegradation and determination of the non-protein amino acid β-cyano-L-alanine, an intermediate of cyanide detoxification in plants
PT6	Alice Pomeislová	Prenylation and Geranylation of Flavonoids for Improved Bioavailability and Anti-Inflammatory Effects
PT7	Waheed Ur Rahman	*Kingella kingae* RtxA toxin interacts with cell surface glycans and disrupts epithelial barrier integrity
PT8	Jan Keil	KhpA/B form a complex with RNase Y as an RNA chaperone in S. *pneumoniae*
PT9	Tomáš Beneš	Linking c-di-AMP homeostasis and cell division regulation in *Streptococcus pneumoniae*
PT10	Ondřej Staněk	EUROTOXIC ACTIVITY OF *BORDETELLA* DERMONECROTIC TOXIN AT SUB-PICOMOLAR CONCENTRATIONS
PT11	Jana Holubová	*Bordetella pertussis* Toxins Drive the Emergence of a Unique $CD8^+$ T Cell Subset in the Respiratory Tract
PT12	Ladislav Bumba	Extreme C-terminus of the FhaB prodomain is essential for interaction of *Bordetella pertussis* with nasal ciliated epithelial cells
PT13	Ludmila Blechová (Brázdilová)	Deciphering the early innate immune response of nasal mucosa to *Bordetella pertussis* infection
PT14	Tania Romero Allsop	The mystery of the *Bordetella* type III secretion system effector protein BteA
PT15	Milada Kambová	Bimodal Behaviour of Intracellular *Salmonella*
PT16	Jan Blumenstein	Cyclic di-GMP signaling and intracellular adaptation of *Aeromonas veronii* in free-living amoebae
PT17	Dominika Luptáková	Molecular Heterogeneity Within Staphylococcal Brain Abscesses
PT18	Tommaso Stefani	Facing the Challenges of Untargeted Metabolomics: Our Path to Workflow Development and Data Integration
PT19	Evgeniya Biryukova	Development of Software for LC-MS Data Processing of Oligonucleotides
PT20	Zuzana Kalaninova	Structural Dynamics of the CyaA Acylated Segment Drive Membrane Invasion: Insights from HDX-MS
PT21	Michael Karpisek	Quantitative Cross-linking Mass Spectrometry Using Data-Independent Acquisition as a Novel Tool for Study of Structural Rearrangement of Proteins in Bacteria
PT22	Nina Nováková	Targeting Biofilm Structure and Integrity with Free and Microparticle-Based Allicin
PT23	Eva Králová	Oil Marbles as SMEDDS-Based Carriers for Enhanced Delivery of Curcumin

PT24	Adéla Brejchová	Glucan Particles as an Innovative Oral Delivery System for Cannabidiol
PT25	Suada Đukaj	Engineering Biosimilar Mucus: Integrating Composition, Rheology and Bioprinting for In Vitro Barrier Model
PT26	Iva Pacáková	The mechanisms that lead to the polarization of microbiota-specific T cells into intraepithelial lymphocytes
PT27	Janaina L. S. Donadio	Western Diet Disrupts Anti-Tumor Immunity and Promotes Melanoma Progression Through Myeloid and T Cell Modulation
PT28	Miloslav Kverka	Oral colistin improves the therapeutic response of tumors to anti-PD1 treatment in an IFN-γ-dependent mechanism
PT29	Veronika Motúzová	Depletion of Myeloid Cells Reduces Tumorigenesis Driven by a High-Protein Diet in a Colitis-Associated Cancer Model
PT30	Maliha Rizwan	Immune Response to Microbial Transglutaminase after polyinosinic-polycytidylic acid (Poly I:C) immunization in a Murine Model
PT31	Halil Mert Solak	The gut microbiome and diet composition of Papua New Guinea Rodents among different altitudes from two mountain ranges
PT32	Leighton J Thomas	Interspecific Divergence and the Effect of Neutral Processes on Microbiota Variation in Inbred Strains Derived from Eight Mouse Species
PT33	Jakub Kreisinger	Making SPF mice great again: Symbiotic Microbiota Restoration in SPF Mice through Dirty Facility Exposure
PT34	Anna Michl	Advancing Gut Microbiome Metabolomics: A Derivatization-Based Approach
PT35	Tomas Hudcovic	Butyrate alters hepatic drug metabolism and expression of short-chain fatty acid transporters and receptors in the colon of mice with DSS-induced colitis
PT36	Dagmar Srutkova	*Bifidobacterium longum* ssp. *longum* CCM 7952-derived peptidoglycan alleviates allergic inflammation through TLR-dependent signaling in a mouse model of airway allergy
PT37	Jan Špaček	Steroids as potential adjuvants: effect on efflux systems and gene expression in *Staphylococcus aureu*
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PT44	Vasilii Shapkin	ALFI: A Global Database of Alien Fungal Introductions
PT45	Petr Baldrian	GlobalFungi - the global atlas of fungal biodiversity
PT46	Tomáš Větrovský	GlobalAMFungi – the global atlas of arbuscular-mycorrhizal fungal biodiversity
PT47	Kateřina Valentová	Interaction of Silymarin Flavonolignans with Human Gut Microbiota
PT48	Martin Krov	confidential
PT49	Dagmar Čížková	Ex germ-free mixe model with natural microbiomes

ABSTRACTS

FT 1-18 – FULL TALKS

FT1

Decoding Resistance: ABCF Proteins Tailor Bacterial Responses to Antibiotic Type and Concentration

Presenter name: **Gabriela Balíková Novotná**

Co-authors: MARKÉTA KOBĚRSKÁ[1], LUDMILA VESELÁ[1,3], MICHAELA NOVOTNÁ[1,3], DURGA MAHOR[1], ANINDA MAZUMDAR[2], NIKOLA PINĎÁKOVÁ[1], PAMELA OMENA PETRAVICIUS[1], JULIE POKORNÁ[1], ZDENĚK KAMENÍK[2]

[1] *Institute of Microbiology of the CAS; BIOCEV, Průmyslová 595, Vestec, Czech Republic*

[2] *Institute of Microbiology of the CAS; Videňská 1083, Prague 4, Czech Republic*

[3] *Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, 12800, Czech Republic*

ABCF-ATPases are increasingly recognized as translation factors that rescue stalled ribosomes when they encounter difficult mRNA templates or are stalled by antibiotics. The latter defines ARE ABCF proteins, known for their role in antibiotic resistance. However, in this study, we reveal a broader role of ARE ABCFs in antibiotic-responsive regulation. Using genetic, OMICs, and biochemical approaches we showed that ARE ABCF proteins TiaA and Are5sc in *Streptomyces coelicolor* use their resistance functions to modulate specialized metabolism and proteosynthesis in response to lincosamide, streptogramin A, and pleuromutilin (LS_AP) antibiotics. Although under LS_AP exposure, either Are5sc or TiaA is essential for activating the biosynthesis of the redox-active antimicrobial actinorhodin, these proteins exhibit distinct functions at the proteome level, defined by their resistance profiles and temporally regulated expression. Are5sc facilitates early adaptive responses by modulating the WblC regulon across a broad range of LS_AP concentrations, while TiaA is induced later, specifically at higher concentrations, where it suppresses antibiotic stress responses, particularly against pleuromutilins. TiaA function thus reflects the ecological context of LS_AP antibiotics as pleuromutilins are produced by fungi, whereas lincosamides/streptogramins originate from actinomycetes. Our findings demonstrate that ARE ABCF proteins, through their resistance function, act as global regulators of translation. This highlights their broader ecological and physiological significance, extending beyond their established role in antibiotic resistance.

Literature: Koběrská,M., Veselá,L., Novotná,M., Mahor,D., Mazumdar,A., Pinďáková,N., Petravicius,P.O., Pokorná,J., Kameník,Z. and Novotná,G.B. (2025) ABCF Protein-Mediated Resistance Shapes Bacterial Responses to antibiotics Based on their Type and Concentration. *mBio* e01568-25.

FT2

From INTERMICRO to medical mycology

Presenter name: **Vladimír Havlíček**

Co-authors: Radim Dobiáš,[1,2] Milan Navrátil,[3,4] Rutuja H. Patil,[5] Dominika Luptáková,[5] David A. Stevens,[6] and Vladimír Havlíček[5]

[1]*Department of Bacteriology and Mycology, National Reference Laboratory for Mycological Diagnostics, Public Health Institute in Ostrava, 702 00 Ostrava, Czechia*
[2]*Institute of Laboratory Medicine, Faculty of Medicine, University of Ostrava, 703 00 Ostrava, Czechia*
[3]*Department of Haemato-oncology, University Hospital Ostrava, Czech Republic, 703 00 Ostrava, Czechia*
[4]*Department of Haemato-oncology, Faculty of Medicine, University of Ostrava, 703 00 Ostrava, Czechia*
[5]*Institute of Microbiology of the Czech Academy of Sciences, 142 00 Prague, Czechia*
[6]*Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA 94305, United States*

We will review the current group activities with a particular focus on collaborations within the INTERMICRO project. Then, we will demonstrate the prospective application of liquid chromatography-mass spectrometry in the diagnosis of a mixed fungal infection. In this study, we compared the performance of chest radiography, galactomannan (sGM), and beta-D-glucan (sBDG) serology with a novel diagnostic method based on creatinine-indexed microbial siderophores in urine. A woman with angioblastic T-cell lymphoma presented with neutropenia following allogeneic transplantation. sGM and sBDG remained positive throughout the 28-day intensive care unit stay. *A. fumigatus* DNA was detected in the induced sputum samples on sampling days 0 and 18. On day 18, a CT scan showed a typical nest sign and *R. microsporus* DNA was detected in sputum. The patient was discharged from the hospital on day 28 and expired seven days later. With our novel strategy based on mass spectrometry, *A. fumigatus* was consistently detected in the urine from day 0 to the end of the stay by the detection of triacetylfusarinine C (uTafC), an A. fumigatus-specific hydroxamate siderophore. An additional invasive *R. microsporus* infection was revealed by the detection of a mucoromycete-specific carboxylate siderophore in urine, rhizoferrin (uRhf), from day seven onwards. Both creatinine-normalized siderophore indices (uTafC/Cr, uRhf/Cr) were sensitive to antifungal therapy, and correlated with fast relapses of the invasive disease in time. This study illustrates how such an early and specific new approach can unravel the complexities of dual fungal infections.

Literature: Dobiáš, R., Navrátil, M., Patil, R. H., Luptáková, D., Stevens, D. A., & Havlíček, V. (2025). Detection of Siderophores as a Superior Noninvasive Diagnostic Tool in Unraveling Mixed Fungal Infections. *ACS omega*, *10*(21), 21908-21914.

FT3

TAILORED GLYCOCONJUGATES DERIVED FROM HUMAN MILK OLIGOSACCHARIDES AND THEIR BIOACTIVITIES

Presenter name: **Pavla Bojarová**

Laboratory of Biotransformation, Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, CZ-142 00 Praha 4, Czech Republic

Human milk and poly-LacNAc oligosaccharides are precious bioactive molecules, hard to prepare by classical synthetic chemistry methods [1]. In the gut, these oligosaccharides can directly control the proliferation of specific bacteria, activity of the host immune system, and epithelial barrier function [2]. Functionalized oligosaccharides may be conveniently produced by telescoping synthesis using tailored enzyme tools - selective mutant and native glycosidases [3] and glycosyltransferases [4]. By enzyme and reaction engineering, the array of possible oligosaccharide products may be extended with glycomimetics, such as in the case of novel *N*,*N*´-diacetyllactosamine-derived glycomimetics prepared using a promiscuous fungal β-*N*-acetylhexosaminidase [5].

We conjugated the prepared oligosaccharides and glycomimetics to biocompatible, bioavailable and non-immunogenic multivalent carriers, *N*-hydroxypropylmethacrylamide (HPMA) copolymer, or serum albumins. The prepared libraries of multivalent glycoconjugates can now be tested for the aims of the INTER-MICRO project in collaboration with other WGs. The prepared glycopolymers have shown immunomodulatory properties in a series of *in vitro* assays in cell cultures. Oligosaccharide-decorated neo-glycoproteins can be immobilized on biosensors and used as bioanalytical tools. The present results highlight the potential of carbohydrate-active enzymes in the preparation of tailored glycoconjugates with a strong biomedical promise.

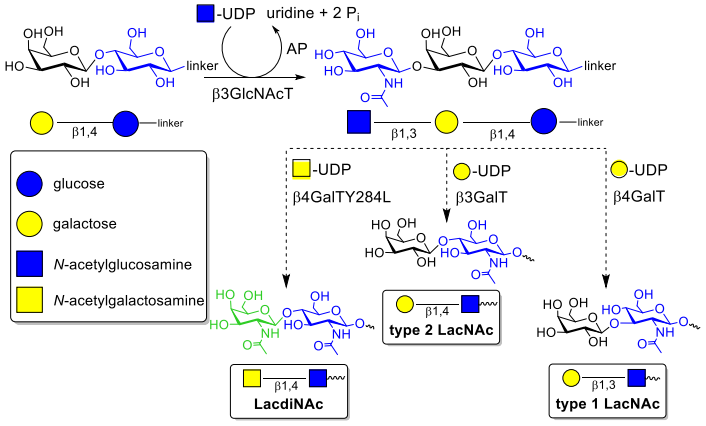

Figure 1. Sample synthesis of tailored bioactive oligosaccharides performed in the project.

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FT4

Calcium-Loaded Acylated Segment Controls the Membrane Penetration Capacity of Repeats in ToXin (RTX) Cytolysins

Presenter name: **Jiri Masin**[1]

Co-authors: Adriana Osickova[1], Zuzana Kalaninova[1,2], Ladislav Bumba[1], Michaela Buresova[1,2], Anna Lesniak[1,2], Joana Filipa Tinoco Marçal[1], David Jurnecka[1], Humaira Khaliq[1], Sascha Vatic[1], Petr Man[1], Petr Novak[1], Radim Osicka[1] and Peter Sebo[1]

[1]*Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*
[2]*Faculty of Sciences, Charles University, Prague, Czech Republic*

The pore-forming Repeats in ToXins (RTX) cytolysins are key virulence factors of many Gram-negative pathogens. Their membrane-penetrating activity depends on fatty-acyl modification of ε-amino groups of one or two conserved lysine residues within an acylated segment that caps a receptor-binding RTX domain. We unraveled the role of two crucial calcium binding sites in the acylated segment that govern the capacity of RTX cytolysins to insert into the target cell membrane, to form pores, and to deliver an N-terminal enzyme domain into target cells. Alterations of these two calcium-binding sites can either ablate or enhance the β_2-integrin receptor-independent membrane penetration capacity of the *Bordetella pertussis* adenylate cyclase toxin (CyaA) and *Escherichia coli* α-hemolysin, thereby setting a new paradigm for membrane interaction by RTX cytolysins.

FT5

Uncovering the Microbial Metabolome Using TimsTOF Mass Spectrometry

Presenter name: **Zdenek Kamenik**[a]

Co-authors: Tommaso Stefani[a], Anna Michl[b], Aninda Mazumdar[a]

[a]*Laboratory of Antibiotic Resistance and Microbial Metabolomics, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*

[b]*Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Novy Hradek, Czech Republic.*

We will present over a year of experience using the TimsTOF HT platform for targeted and untargeted metabolomics to explore the chemical diversity of microbial systems. Our talk will highlight the range of biological matrices and metabolite classes we can analyze, including current limitations of the technology. We will also outline our data analysis pipeline combining commercial and open-source tools for feature table assembly, metabolite annotation using spectral libraries and in silico tools, and molecular networking for chemical space visualization with metadata mapping. Finally, we will discuss the potential of repository-scale metabolomics and the importance of public data sharing for advancing microbial metabolome research.

FT6

Deciphering host-derived signals that modulate c-di-GMP in *Bordetella*

Presenter name: **Denisa Vondrova**

Co-authors: Barbora Chvatalova, Matija Perovic, Jana Kamanova

Laboratory of Infection Biology, Institute of Microbiology, Czech Academy of Sciences, Videnska 1083, Prague 142 00, Czech Republic

Classical *Bordetella* species cause respiratory infections in humans and animals, with *B. pertussis* responsible for whooping cough in humans and *B. bronchiseptica* primarily associated with chronic infections in various mammals. During the colonization of the host respiratory tract, bordetellae form biofilms on the airway mucosa and regulate the expression of virulence factors, including the type three secretion system (T3SS). Both biofilm formation and T3SS activity are controlled by the second messenger bis-(3'-5')-cyclic-dimeric guanosine monophosphate (c-di-GMP). However, the environmental signals and regulatory pathways that control c-di-GMP levels in bordetellae are still poorly understood. Our work aims to identify environmental factors that modulate intracellular c-di-GMP levels in *B. bronchiseptica* and *B. pertussis*.

Using a genetically encoded fluorescence-based c-di-GMP biosensor in combination with flow cytometry, we screen a panel of approximately 400 small molecules for their impact on intracellular c-di-GMP levels. The hits from the first screening will be validated by fluorescence microscopy. In the next phase, we will use targeted gene deletions and a high-density transposon mutant library in combination with transposon insertion sequencing (Tn-seq) to identify specific diguanylate cyclases, phosphodiesterases and sensory modules responsible for these responses. By uncovering the environmental and genetic factors that regulate c-di-GMP signaling, we aim to gain insight into the control of virulence and persistence in *Bordetella* species.

FT7

Perspectives in Proteomics: Picogram-Level Proteomics Workflows

Presenter name: Saša Vatić [1]

Co-authors: Petr Novák[1], Michaela Burešová[1,2], Jiri Mašín[1], Peter Sebo[1]

[1] *Institute of Microbiology of the CAS, Prague 4, Czech Republic;*

[2] *Faculty of Science, Charles University, Prague 2, Czech Republic*

Recent advances in proteomics have enabled picogram-level sensitivity for the analysis of complex biological samples, opening new opportunities for structural biology. Low-input workflows have been optimized using Evosep Whisper ZOOM gradients (80 SPD) in combination with IonOpticks Aurora zero-dead-volume nanoflow columns, achieving exceptional chromatographic reproducibility and resolution. Automated on-tip digestion and sample preparation pipelines have been implemented to minimize handling losses and enhance throughput. The timsTOF SCP platform, operated under DDA and DIA acquisition strategies and coupled with DIA-NN for automated data processing, has demonstrated an excellent sensitivity. Whole-cell lysates at 40 pg yielded over 2,000 protein groups, while acylated peptides were identified at inputs as low as 5 pg. The integration of Evosep's High-Organic gradients (100 SPD) has revolutionized the separation of hydrophobic peptides in bottom-up workflows.

These capabilities have facilitated the characterization and quantification of protein acylation, revealing site-specific modification patterns. Ion-mobility separation combined with high-resolution chromatography has enabled the detection of low-abundance and structurally heterogeneous peptides, addressing key challenges in PTM analysis.

Integration of emerging methods such as diagonal-PASEF, real-time online searches (ProteoScape, DIA-NN), and complementary structural techniques is anticipated. Synergies with super-resolution microscopy, oxidative labeling, HDX-MS, and crosslinking-MS promise multi-dimensional, in situ characterization of molecular assemblies within their native biological context.

FT8

Advanced Encapsulation Strategies for Various Biological Entities and Biomolecules: From Spray Drying to Microfluidic Applications

Presenter name: **Viet Tomáš Nguyen**

Co-authors: Nina Nováková, Viet Tomáš Nguyen, Alina Mamedova, Romana Hupková, Lucie Mašková, Ondřej Kašpar

Department of Chemical Engineering, University of Chemistry and Technology, Prague

In this study, we present various methods for the encapsulation of single molecules and cells. In the first part, the encapsulation of precursors involved in the enzymatic reaction will utilize the spray drying technique, with an option for employing either a two-fluid or a three-fluid nozzle. The choice of nozzle significantly influences the final composition and architecture of the dried powder particles, resulting in a matrix type from the two-fluid nozzle and a core-shell type from the three-fluid nozzle. We will discuss the process parameters and material selection necessary to achieve the most effective antibacterial outcomes.

In the second part, we will introduce methods for cell encapsulation and single-cell analysis, focusing on the use of microfluidic and millifluidic devices. We will explore stop-flow lithography, a technique that utilizes photocrosslinkable polymers to create micro-robots designed for cell encapsulation, capable of responding to external stimuli and providing, for example, mechanostimulation testing. Furthermore, we will discuss strategies for cell entrapment and interaction studies using microfluidics, including investigations of cell-surface interactions, cell-particle dynamics, and multi-species interactions.

FT9

Overview and Applications of Drug Delivery Systems for Inter-Species Communication and Signaling

Presenter name: **František Štěpánek**

Department of Chemical Engineering, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague 6, Czech Republic

Research on drug delivery systems is generally motivated by the desire to control the temporal, spatial, and concentration profile of therapeutically active molecules in the human body. The aim is to deliver a bioactive payload to a specific organ or tissue at therapeutic concentrations without exposing the rest of the body to unnecessarily high levels of these compounds, which can often be cytotoxic or exhibit undesirable side effects when not targeted. The desire to avoid non-specific spreading and dilution of the bioactive compound throughout the body is motivated not only by toxicity concerns but also to minimize opportunities for the development of drug-resistance. The design of an effective drug delivery system faces several challenges such as pH-dependent solubility of the drug, water-lipid partitioning, permeation across biomembranes, or enzymatic degradation of the drug. Encapsulation of the drug or prodrug into a suitable carrier particle is often a way of overcoming some of these challenges. Signaling within microbial communities or between microbes and eucaryotic hosts faces very similar problems: the signaling substance must reach the target at a sufficiently high local concentration to transmit the message it is carrying, all the while passing through a complex microenvironment that contains both aqueous and lipidic phases, gradients of pH and/or ionic strength, various barriers in the forms biomembranes, and a plethora of intra- and extra-cellular enzymes that can degrade the signaling species. This talk will introduce our recent work on the design and application of drug delivery systems – specifically liposomes, drug nanocrystals, and porous carriers – in the context of their potential use as tool for the investigation of communication with microbial communities or between microbes and eucaryotic hosts.

FT10

A new generation of *in vitro* intestinal barrier models for evaluating the effects of drugs and food ingredients

Presenter name: **Jitka Viktorová**

Co-authors: Kučerová Emílie, Strnad Ondřej, Nejedlý Tomáš, Zahradníček Richard

Department of Biochemistry and Microbiology, University of Chemistry and Technology in Prague, Technická 5, 166 28 Prague

The intestinal barrier is crucial for nutrient absorption, protection from pathogens and maintenance of immune balance. Disruption of this balance leads to serious diseases such as inflammatory bowel disease (IBD), colorectal cancer (CRC) or infections caused by pathogenic bacteria such as *Clostridium difficile*. Current approaches to treating IBD and CRC have a number of limitations, including the need for long-term antibiotics and immunosuppressants, or the development of resistance to chemotherapy and the formation of metastases, which have a number of socio-economic implications. The development of advanced *in vitro* models of the intestinal barrier is therefore essential not only for ethical principles but also for a better understanding of the mechanisms of intestinal homeostasis and the effects of biologically active agents.

The aim of this work is the development of advanced *in vitro* models of the intestinal barrier to study interactions between the gut microbiota and host cells under physiological and pathological conditions, namely: (i) an intestinal barrier populated by physiological microbiota; (ii) an intestinal barrier affected by pathological microbiota; (iii) chronic inflammatory conditions; and (iv) organoids derived from colorectal cancers.

These models will allow to test the effect of different substances on the state of the intestinal barrier and on pathological conditions including IBD and CRC. The results will contribute to a deeper understanding of the roles of the gut microbiota and barrier in human health and disease.

FT11

WP3 overview Who, what and with whom (Martin Schwarzer) – NO ABSTRACT

FT12

Segmented filamentous bacteria (SFB)-Induced Intraepithelial Lymhocytes Differentiation: The Critical Molecular and Cellular Players

Presenter name: **Jan Dobeš**

Co-authors: Iva Pacáková[1], Anna Michl[2], Katarína Kováčová[1], Tomáš Brabec[1], Martin Schwarzer[2], Jan Dobeš[1]

[1]*Laboratory of Microbial Immunology, Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic*

[2]*Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Nový Hrádek, Czech Republic*

Segmented filamentous bacteria (SFB) are anaerobic, spore-forming, Clostridia-like microbes. Their adhesion triggers a strong intestinal immune response, characterized by Th17 cell accumulation and increased IgA secretion by plasma cells. Recently, we reported that naïve $CD4^+$ T cells reactive to SFB can also differentiate into induced intraepithelial lymphocytes and express cytotoxic molecules. In parallel, the presence of SFB induces upregulation of Th1-derived IFN-γ, which in turn leads to a significant increase in MHCII expression on intestinal epithelial cells. The accumulation of SFB-specific intraepithelial lymphocytes is markedly reduced when MHCII expression on intestinal epithelial cells is ablated.

We investigated whether $CD4^+$ SFB-specific T cells become intraepithelial lymphocytes immediately upon activation or if already activated and polarized cells later adopt the IEL fate. Our findings indicate that SFB-specific Th1 cells are particularly efficient in generating SFB-reactive intraepithelial lymphocytes. Using genetic ablation models, we further revealed that this process depends on conventional dendritic cells.

Additionally, we explored other cellular players and molecular signals involved in the gut tissue adaptation of SFB-specific intraepithelial lymphocytes. This includes $CSF1R^+$ myeloid cells, which play a key role in regulating the acquisition of gut-residency markers such as CD8αα and enhancing granzyme B expression in SFB-specific intraepithelial lymphocytes. Moreover, IFN-γ signaling is essential for their full gut tissue adaptation, as its disruption impairs this process.

These findings contribute to a deeper understanding of host–gut microbiota interactions and the cellular mechanisms governing the unconventional transition of $CD4^+$ T cells into cytotoxic intraepithelial lymphocytes.

FT13 (+ PT26)

The mechanisms that lead to the polarization of microbiota-specific T cells into intraepithelial lymphocytes

Presenter name: **Iva Pacáková**[1]**,** Anna Michl[2]

Co-authors: Katarína Kováčová[1], Tomáš Brabec[1], Martin Schwarzer[2], Jan Dobeš[1]

[1]*Laboratory of Microbial Immunology, Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic*
[2]*Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Nový Hrádek, Czech Republic*

Segmented filamentous bacteria (SFB) are Gram-positive, clostridia-like commensal bacteria that have evolved a unique life strategy involving close interaction with the host epithelial cells of the small intestine. Under homeostatic conditions, SFB remain non-pathogenic and they are controlled by the immune system – primarily through Th17 cell response – which prevents them from penetrating the epithelial barrier and causing pathological inflammation.
While the Th17-mediated immune response to SFB has been well characterized, other resonses induced by SFB in the host intestine remain poorly understood. Our recent findings revealed conversion of SFB-specific CD4+ T cells into granzyme-expressing intraepithelial lymphocytes (IELs). Likewise, Treg-inducing bacteria such as Lactobacillus reuteri exhibit a similar capacity to induce IELs. However, it is unclear if and how these bacteria cooperate together in the induction of IELs. Therefore, we aim to determine, how SFB and L.reuteri contribute to the IELs induction, elucidate the differences in the origin and immune mechanisms driving SFB- and L. reuteri-induced IELs differentiation and assess how these mechanisms influence IELs phenotype.

See also PT26

FT14

Microbiome gut-brain axis Introduction (**Petra Procházková**) – NO ABSTRACT

FT15

Growth-promoting properties of lactic acid bacteria

Presenter name: **Tereza Novotná**

Co-authors: Veronika Drgoňová, Umesh Gautam, Sudhanshu Sherkar, Barbora Valášková, Tereza Horníková, Dagmar Šrůtková, Martin Schwarzer

Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Novy Hradek, Czech Republic

The ability of *Lactiplantibacillus plantarum* WJL bacteria to support the growth of gnotobiotic invertebrate and vertebrate hosts in models of chronic malnutrition is known and well-established (Storelli et al, 2011, Schwarzer et al., 2016). Whether other bacteria from different genera exert the growth-promoting capability is currently unknown. We selected two different bacterial strains from two different Bifidobacteria species and tested them in a gnotobiotic mouse model of chronic undernutrition.

Parental generation of germ-free (GF) C57BL/6J mice was monocolonized with one of these four bifidobacterial strains: *Bifidobacterium longum ssp. longum* (Bl367 and Bl372) and *Bifidobacterium adolescentis* (Bad368 and Bad373). Male mice from the filial generation were weaned at day 21 after birth on an experimental low-protein, low-fat (MAL) diet. Their growth was monitored weekly by measuring body length and weight for 5 weeks.

Mice monocolonized with *Bifidobacterium longum* strains (most prominently Bl372 strain) showed improved systemic growth compared to mice monocolonized with *Bifidobacterium adolescentis* strains or the control GF group. This was accompanied by elevated levels of IGF-1 in the serum. Histological analysis of the small intestine revealed significantly longer intestinal villi of Bl372 monocolonized mice. Bacterial load in the gut was reduced in all mice on MAL diet compared to breeding diet-fed controls. Bulk RNA sequencing of jejunal tissue revealed differences in gene expression.

Conclusion: Our results show that selected *Bifidobacterium longum* 372 strain has the ability to support juvenile growth upon nutritional challenge. The ability to support growth differs among *Bifidobacteria* and is species- as well as strain-specific.

Literature:

Storelli et al. “Lactobacillus plantarum promotes Drosophila systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing.” *Cell metabolism* 2011. doi:10.1016/j.cmet.2011.07.012

Schwarzer et al. “Lactobacillus plantarum strain maintains growth of infant mice during chronic undernutrition.” *Science* 2016 doi:10.1126/science.aad8588

FT16

Bacterial populations active during lignin depolymerization

Presenter name: **Ana Catalina Lara**

Co-authors: Magdalena Folkmanová (1), Michal Strejček (1), Miluše Hradilová (2), Michal Kolář (2), Tomáš Cajthaml (3), Petr Pajer (4), Ondřej Uhlík (1)

(1) Department of Biochemistry and Microbiology, Faculty of Food and Biochemical. Technology, University of Chemistry and Technology, Prague, Czech Republic
(2) Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
(3) Institute of Microbiology of the CAS, Prague, Czech Republic
(4) Military Health Institute, Ministry of Defence of the Czech Republic, Praha, Czech Republic

Lignin is the most abundant and chemically prolific resource in nature. The effective and comprehensive utilization of lignin has become an important topic with the advent of global politics and industrial practices into being more sustainable, and less oil dependent. Understanding how microorganisms transform lignin into potentially interesting added-value products, and identifying key enzymes is not new, however, exploring the depolymerization potential of bacterial populations and shifting the focus in bacteria beyond the funneling pathways is a recent development.

In this work, we identified active bacterial populations from lignin-degrading enriched garden soil using a combination of isotope probing with pure lignin and metagenomics. The results showed that a diversity of bacterial populations are active during the depolymerization of lignin, pointing to the participation of bacterial population in the active degradation of lignin in nature.

FT17

Towards a relevant experimental model of arbuscular mycorrhizal fungal hyphosphere

Presenter name: **Valeriia Belova**

Co-authors: Hana Hršelová, Martin Rozmoš, Jan Jansa

Lab of Fungal Biology, Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, 14200 Praha 4, Czech Republic

Arbuscular mycorrhizal fungi (AMF) are important plant symbionts that significantly contribute to plants' acquisition of nitrogen (N) and phosphorus (P) from the remote soil patches. However, they cannot utilize nutrients bound in organic compounds, such as chitin and phytate, due to their limited exoenzymatic capabilities. Thus, they depend on microorganisms inhabiting the vicinity of their hyphae, the hyphosphere, to acquire N and P from organic compounds. Despite their important role in mineral nutrient cycling, knowledge about the interactions between AMF and hyphospheric microbes and their impact on soil-plant nutrient fluxes is still limited.

To better understand the subject, we attempted to establish a relevant and manageable model of the AMF hyphosphere. A few dozen bacterial strains were isolated from this enigmatic soil zone and identified through 16S sequencing. Isolates have been screened for their ability to degrade chitin, phytate, and/or proteins. 24 taxonomically and functionally non-redundant isolates were evaluated for possible antagonism and subsequently tested *in vitro* for their interactions with actively growing AMF. Afterward, three synthetic communities (SynComs) were proposed and tested for their mineralization efficiency in the presence of AMF and ^{15}N-labeled chitin in solidified liquid and artificial soil environments.

Eleven isolates migrated along the AMF hyphae, and two positively affected fungal sporulation. Analyses demonstrated SynCom-dependent differences in N concentration in hyphae and roots. Analysis of the resulting SynCom communities (post incubation) is currently being analyzed Preliminary results suggest the potential to generate a suitable model and address open questions in AMF hyphosphere ecophysiology.

FT18

Harnessing global databases to understand fungal ecology: lessons from GlobalFungi and GlobalAMFungi

Presenter name: **Petr Kohout**

Co-authors: Tomáš Větrovský[1], Inaki Odriozola[1], Felix Wesener[1], Florian Barbi[1], Zuzana Kolaříková[2], Petr Baldrian[1]

[1]Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, CZ-142 20 Prague, Czech Republic
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GlobalFungi and GlobalAMFungi databases are open sources and open access initiatives that compile the most comprehensive atlases of fungal taxa distribution, so far. Altogether these databases provide almost 100,000 samples of fungal communities from all continents and environments and source materials, which were analysed using next-generation sequencing technologies. Besides the applicability of such dataset to study biogeography and global diversity patterns of fungi, it also offers unique opportunity to better understand ecology of individual fungal species. In this talk I would like to present recent advances in understanding global diversity patterns of various fungal ecological groups. I will show different importance of host plant distribution of global diversity patterns of arbuscular mycorrhizal, ectomycorrhizal and ericoid mycorrhizal fungi. Besides that, I will demonstrate the applicability of GlobalFungi and GlobalAMFungi databases to identify various fungal traits and their applicability to better understand assembly rules structuring fungal communities. Recently, we identified preferential investment of arbuscular mycorrhizal fungal taxa into intraradical vs. extraradical biomass. In subsequent application, we found higher occurrence of arbuscular mycorrhizal fungal taxa with preferential investment to soil biomass on sites with low levels of nutrient concentrations, while arbuscular mycorrhizal fungal taxa with preferential investment to root biomass dominated in highly fertile soils. Altogether, GlobalFungi and GlobalAMFungi databases proved to be unprecedented source of data with potential to significantly improve our understanding of fungal ecology and biology.

FT 19 – 23 – PRESENTATION OF SELECTED PAPERS FROM THE PROJECT

FT19

New bacterial aryl sulfotransferases: Effective tools for sulfation of polyphenols.

Presenter name: **Barbora Petránková**

Brodsky, K., Petránková, B., Petrásková, L., Pelantová, H., Kren, V., Valentová, K., & Bojarová, P. (2024). New bacterial aryl sulfotransferases: Effective tools for sulfation of polyphenols. *Journal of Agricultural and Food Chemistry*, *72*(40), 22208-22216.

The preparation of pure metabolites of bioactive compounds, particularly (poly)phenols, is essential for the accurate determination of their pharmacological profiles *in vivo*. Since the extraction of these metabolites from biological material is tedious and impractical, they can be synthesized enzymatically *in vitro* by bacterial PAPS-independent aryl sulfotransferases (ASTs). However, only a few ASTs have been studied and used for (poly)phenol sulfation. This study introduces new fully characterized recombinant ASTs selected according to their similarity to the previously characterized ASTs. These enzymes, produced in *Escherichia coli*, were purified, biochemically characterized, and screened for the sulfation of nine flavonoids and two phenolic acids using *p*-nitrophenyl sulfate. All tested compounds were proved to be substrates for the new ASTs, with kaempferol and luteolin being the best converted acceptors. ASTs from *Desulfofalx alkaliphile* (*Dal*AST) and *Campylobacter fetus* (*Cf*AST) showed the highest efficiency in the sulfation of tested polyphenols. To demonstrate the efficiency of the present sulfation approach, a series of new authentic metabolite standards, regioisomers of kaempferol sulfate, were enzymatically produced, isolated, and structurally characterized.

FT20

Protists are key players in the utilization of protein nitrogen in the arbuscular mycorrhizal hyphosphere

Presenter name: **Anukool Vaishnav**

Vaishnav, A., Rozmoš, M., Kotianová, M., Hršelová, H., Bukovská, P., & Jansa, J. (2025). Protists are key players in the utilization of protein nitrogen in the arbuscular mycorrhizal hyphosphere. *New Phytologist*, *246*(6), 2753-2764.

While largely depending on other microorganisms for nitrogen (N) mineralization, arbuscular mycorrhizal fungi (AMF) can transfer N from organic sources to their host plants. Here, we compared N acquisition by the AMF hyphae from chitin and protein sources and assessed the effects of microbial interactions in the hyphosphere.

We employed in vitro compartmented microcosms, each containing three distinct hyphosphere compartments amended with different N sources (protein, chitin, or ammonium chloride), one of which was enriched with 15N isotope. All hyphosphere compartments were supplied with *Paenibacillus* bacteria, with or without the protist *Polysphondylium pallidum*. We measured the effect of these model microbiomes on the efficiency of 15N transfer to roots via the AMF hyphae.

We found that the hyphae efficiently took up N from ammonium chloride, competing strongly with bacteria and protists. Mobilization of 15N from chitin and protein was facilitated by bacteria and protists, respectively. Notably, AMF priming significantly affected the abundance of bacteria and protists in hyphosphere compartments and promoted mineralization of protein N by protists. Subsequently, this N was transferred into roots.

Our results provide the first unequivocal evidence that roots can acquire N from proteins present in the AMF hyphosphere and that protists may play a crucial role in protein N mineralization.

FT21

The Bordetella effector protein BteA induces host cell death by disruption of calcium homeostasis

Presenter name: **Jana Kamanova**

Zmuda, M., Sedlackova, E., Pravdova, B., Cizkova, M., Dalecka, M., Cerny, O., … & Kamanova, J. (2024). The Bordetella effector protein BteA induces host cell death by disruption of calcium homeostasis. *Mbio*, *15*(12), e01925-24.

Bordetella pertussis is the causative agent of whooping cough in humans, a disease that has recently experienced a resurgence. In contrast, *Bordetella bronchiseptica* infects the respiratory tract of various mammalian species, causing a range of symptoms from asymptomatic chronic carriage to acute illness. Both pathogens utilize type III secretion system (T3SS) to deliver the effector protein BteA into host cells. Once injected, BteA triggers a cascade of events leading to caspase 1-independent necrosis through a mechanism that remains incompletely understood. We demonstrate that BteA-induced cell death is characterized by the fragmentation of the cellular endoplasmic reticulum and mitochondria, the formation of necrotic balloon-like protrusions, and plasma membrane permeabilization. Importantly, genome-wide CRISPR-Cas9 screen targeting 19,050 genes failed to identify any host factors required for BteA cytotoxicity, suggesting that BteA does not require a single nonessential host factor for its cytotoxicity. We further reveal that BteA triggers a rapid and sustained influx of calcium ions, which is associated with organelle fragmentation and plasma membrane permeabilization. The sustained elevation of cytosolic Ca^{2+} levels results in mitochondrial calcium overload, mitochondrial swelling, cristolysis, and loss of mitochondrial membrane potential. Inhibition of calcium channels with 2-APB delays both the Ca^{2+} influx and BteA-induced cell death. Our findings indicate that BteA exploits essential host processes and/or redundant pathways to disrupt calcium homeostasis and mitochondrial function, ultimately leading to host cell death.

FT22

Genetic background and microbiome drive susceptibility to epicutaneous sensitization and food allergy in adjuvant-free mouse model

Presenter name: **Dagmar Šrůtková**

Hornikova, T., Jelinkova, A., Jiraskova Zakostelska, Z., Thon, T., Coufal, S., Polouckova, A., ... & Srutkova, D. (2025). Genetic background and microbiome drive susceptibility to epicutaneous sensitization and food allergy in adjuvant-free mouse model. *Frontiers in Immunology*, *15*, 1509691.

Background: The dual allergen exposure hypothesis states that sensitization to food antigens occurs through a damaged skin barrier in individuals with no previous oral tolerance to certain foods. However, the resulting allergic reaction could depend on factors such as the host's genetic predisposition as well as the skin and gut microbiota.

Methods: Specific-pathogen-free BALB/c and C57BL/6 and germ-free (GF) BALB/c mice were epicutaneously sensitized with ovalbumin (OVA) via dorsal tape-stripped skin and challenged with OVA by intragastric gavage. The development of food allergy (FA) symptoms, the Th2 and mast cell immune response and differences in the skin and gut microbiota were investigated.

Results: BALB/c mice, but not C57BL/6 mice, showed severe clinical signs of FA (hypothermia, diarrhea) as well as a stronger serum antibody response and Th2 cytokine secretion in the spleen and jejunum after OVA-treatment. The increased mast cell count correlated with higher MCPT-1 production and histidine decarboxylase mRNA expression in the jejunum of these mice. The 16S rRNA sequencing analysis revealed lower abundance of short-chain fatty acids producing bacteria in the gut microbiome of OVA-treated BALB/c mice. Changes in the β-diversity of the gut microbiome reflect both the genetic background as well as the OVA treatment of experimental mice. Compared to SPF mice, GF mice developed more severe anaphylactic hypothermia but no diarrhea, although they had a higher mast cell count, increased MCPT-1 production in the jejunum and serum, and increased arachidonate 5-lipoxygenase mRNA expression.

Conclusions: We show that the BALB/c mice are a mouse strain of choice for model of adjuvant-free epicutaneous sensitization through the disrupted skin barrier and following food allergy development. Our results highlight the significant influence of genetic background and microbiota on food allergy susceptibility, emphasizing the complex interplay between these factors in the allergic response.

FT23

The Actinobacillus pleuropneumoniae apxIV operon encodes an antibacterial toxin-immunity pair.

Presenter name: **Ladislav Bumba**

Slivenecka, E., Jurnecka, D., Holubova, J., Stanek, O., Brazdilova, L., Cizkova, M., & Bumba, L. (2025). The Actinobacillus pleuropneumoniae apxIV operon encodes an antibacterial toxin-immunity pair. *Microbiological Research*, *292*, 128043.

The ApxIVA protein belongs to a distinct class of a "clip and link" activity of Repeat-in-ToXin (RTX) exoproteins. Along with the three other pore-forming RTX toxins (ApxI, ApxII and ApxIII), ApxIVA serves as a major virulence factor of Actinobacillus pleuropneumoniae, the causative agent of porcine pneumonia. The gene encoding ApxIVA is located on a bicistronic operon downstream of the orf1 gene and is expressed exclusively under in vivo conditions. Both ApxIVA and ORF1 are essential for full virulence of A. pleuropneumoniae, but the molecular mechanisms by which they contribute to the pathogenicity are not yet understood. Here, we provide a comprehensive structural and functional analysis of ApxIVA and ORF1 proteins. Our findings reveal that the N-terminal segment of ApxIVA shares structural similarity with colicin M (ColM)-like bacteriocins and exhibits an antimicrobial activity. The ORF1 protein resembles the colicin M immunity protein (Cmi) and, like Cmi, is exported to the periplasm through its N-terminal signal peptide. Additionally, ORF1 can protect bacterial cells from the antimicrobial activity of ApxIVA, suggesting that ORF1 and ApxIVA function as an antibacterial toxin-immunity pair. Moreover, we demonstrate that fetal bovine serum could elicit ApxIVA and ORF1 production under in vitro conditions. These findings highlight the coordinated action of various RTX determinants, where the fine-tuned spatiotemporal production of ApxIVA may enhance the fitness of A. pleuropneumoniae, facilitating its invasion to a resident microbial community on the surface of airway mucosa.

LT 1-8, 13, 14 - LIGHTNING TALKS WITH POSTER PRESENTATIONS

LT1 – see also PT1

EXPLORING THE ANTIBIOTIC-RESPONSIVE REGULATORY FUNCTION OF ABCF PROTEINS IN ANTIBIOTIC-PRODUCING BACTERIA

Presenter name: **Mufarrah Mehboob**

Co-authors: MARKÉTA KOBĚRSKÁ, ANINDA MAZUMDAR, ZDENĚK KAMENÍK AND GABRIELA BALÍKOVÁ NOVOTNÁ*

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ABCF proteins are cytosolic ATPases of the ABC superfamily, which includes proteins involved in the regulation of translation or the protection of the ribosome against antibiotics. Despite their different biological functions, all characterized ABCFs bind to the same site on the ribosome and modulate the peptidyl transferase center. However, the vast majority of the 30 bacterial ABCF subfamilies have not yet been characterized. Our recent findings have shown that one of the ABCFs, LmrC, induces lincomycin biosynthesis in response to lincosamide antibiotic added to the medium by activating transcription of cluster situated transcriptional regulator *lmbU*[1]. However, it is not known how widespread ABCF-mediated antibiotic signaling in antibiotic-producers is.

Bioinformatic search revealed several ABCF proteins encoded within BGCs for ribosome-binding antibiotics. Furthermore, the type of ABCF protein encoded in a particular BGC correlates with the mode of antibiotic binding to the ribosome. We hypothesize that these ABCF proteins participate in signaling cascades that initiate antibiotic production in response to the presence of antibiotics similar to LmrC. For the study, we have already selected candidate strains containing representatives of the ARE4, ARE5, AAF1, AAF2 and AAF4 subfamilies of ABCF proteins. We will identify regulatory genes, that are directly regulated by ABCF proteins and further characterize ABCF signaling in heterologous host. Studying these biosynthetic pathways reveals fundamental mechanisms of antibiotic-mediated signaling in soil and may, in the future, enable the engineering of strains capable of producing novel antibiotics or enhancing the yield of existing ones.

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LT2 see also PT2

A BIOINFORMATIC APPROACH FOR THE CHARACTERIZATION OF MICROBIAL SIDEROPHORES FROM LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY DATASETS

Presenter name: **Jiří Novák**

Co-authors: Dominika Luptáková, Vladimír Havlíček

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Liquid chromatography-mass spectrometry (LC-MS) is a widely used analytical technique for the characterization of microbial metabolites in biological samples. Siderophores are iron-carrying molecules that can serve as markers of infectious diseases caused by microorganisms and can be well studied by LC-MS. Here, we present a bioinformatic approach for the characterization of siderophores from LC-MS datasets that can be split into two parts. First, we show how the concentrations of siderophores can be monitored in the kinetics of *in vitro* experiments. Second, our improved method for the characterization of novel siderophores from high-resolution mass spectra using a database-free approach is discussed from the perspective of annotation accuracy and speed. A showcase dataset of mass spectra corresponding to *in vitro* samples inoculated with *Aspergillus fumigatus* was used. The supernatant containing fungal siderophores was collected from 12 to 72 hours postinoculation. High-resolution mass spectra were recorded using a 12T SolariX FT-ICR mass spectrometer in positive ion mode with electrospray ionization. The concentrations of ferriforms of triacetylfusarinine B/C and ferricrocin were determined. The discussed methods have been incorporated into our in-house and open-source tool CycloBranch.

LT3 - see also PT4

NOVEL FUNGAL α-l-FUCOSIDASE EXHIBITING TRANSFUCOSYLATION ACTIVITY

Presenter name: **Pavlína Nekvasilová**[1,2]

Co-authors: Michaela Glozlová[1], Andrea Vopálenská[1,2], Vladimír Křen[1], Pavla Bojarová[1]

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α-L-Fucosidases of glycoside hydrolase family 29 (GH29; CAZy; http://www.cazy.org/) are retaining *exo*-glycosidases that typically hydrolyze α-linked fucose from non-reducing end of polysaccharides and glycoconjugates [1, 2]. GH29 α-L-fucosidases have a broad substrate specificity, hydrolyzing α-(1→2), α-(1→3), α-(1→4) and α-(1→6) linked fucosyl residues from glycans [3]. Of the two GH29 subfamilies with distinct sequence homology and substrate specificity, GH29A enzymes are more suitable for transfucosylation because they utilize chromogenic substrates such as 4-nitrophenyl α-L-fucopyranoside (*p*NP-Fuc) or 2-chloro-4-nitrophenyl α-L-fucopyranoside (CNP-Fuc). To date, the CAZy database contains 14 527 annotated GH29 α-l-fucosidases, only four of which belong to the fungal kingdom and one of them is fully characterized [4].

We present here the screening of selected fungi potentially producing proteins with α-l-fucosidase activity under the induction by six carbohydrate inducers. We have identified new fungal α-l-fucosidases and produced them heterologously in a *Pichia pastoris* expression system. We identified their transglycosylation capabilities with *p*NP-Fuc donor.

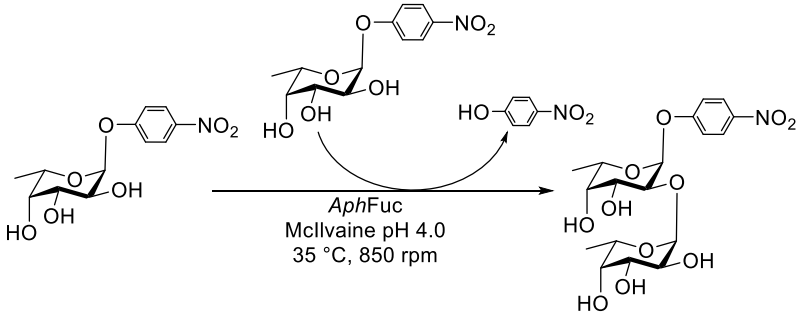

Figure 1. Transglycosylation reaction catalyzed by a novel fungal α-L-fucosidase.

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LT4 - see also PT7

Kingella kingae RtxA toxin interacts with cell surface glycans and disrupts epithelial barrier integrity

Presenter name: **Waheed Ur Rahman**

Co-authors: Adriana Osičková[1], Radovan Fišer[2], Peter Šebo[1] & Radim Osička[1].

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abstract confidential

LT5 - see also PT8

KhpA/B form a complex with RNase Y as an RNA chaperone in S. *pneumoniae*

Presenter name: **Jan Keil[1,2]**

Co-authors: Aleš Ulrych[1], Václava Stauberová[1,2], Karolina Buriánková[1], Linda Doubravová[1], Petr Man[3], Alan Kádek[3], Tomáš Smrčka[3] and Pavel Branny[1]

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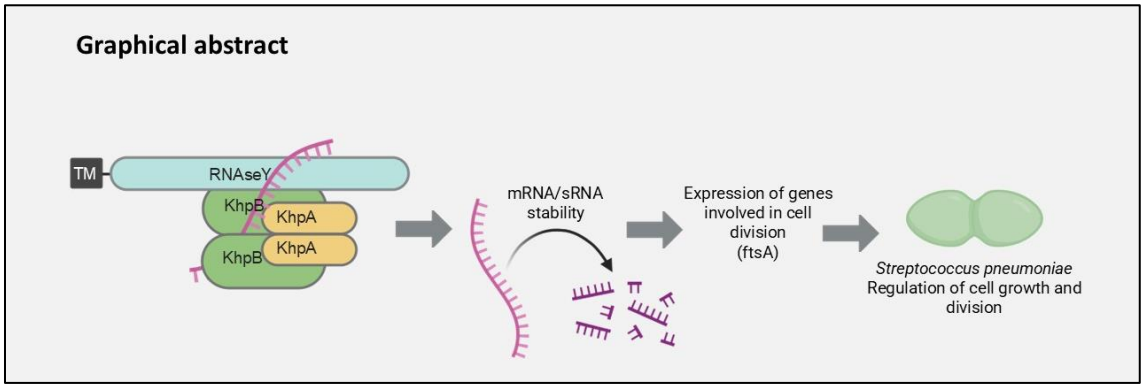

KhpB, a substrate for StkP kinase and Php phosphatase, regulates various cellular processes, particularly cell division in S. pneumoniae. It forms a complex with KhpA, which possesses a single RNA-binding KH domain. KhpB is a multidomain protein featuring the essential Jag_N domain for septal localization, a linker region that contains the primary phosphorylation site T89, and two RNA-binding domains (KH and R3H) at the C-terminus. While the RNA chaperone functions are suggested, the specific roles of these domains remain unclear. To investigate these functions, we created truncated versions of KhpB based on its domains and characterized them using co-immunoprecipitation, mass spectrometry, BACTH assays, microscopy, and immunodetection. Our analyses revealed that the Jag_N domain interacts with various proteins involved in cell division and cell wall synthesis, underscoring its role in septal localization. The C-terminal RNA-binding motifs facilitate interactions with KhpA and RNA processing proteins, highlighting their significance in RNA metabolism. RNase Y, an endonuclease linked to cell division, metabolism, stress response, and virulence, was identified as a strong interaction partner of the KhpA/B complex, indicating a close functional relationship. We propose that KhpA/B forms a multimeric complex with RNase Y, potentially acting as an RNA chaperone to regulate RNA critical for bacterial physiology. Further experimentation will explore the significance of this complex in RNA regulation and bacterial pathogenicity.

LT6 - see also PT17

Molecular Heterogeneity Within Staphylococcal Brain Abscesses

Presenter name: **Dominika Luptáková[1]**

Co-authors: Kyle T. Enriquez[2,3], Martin Dufrense[4,5], Madeline Colley[4,5], Jesse P.Y. Chen[2,3], Itidal Reslane[2,3], Jacqueline Van Ardenne[4,6], Eric P. Skaar[2,3], Jeffrey M. Spraggins[2,4,5,6]

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Central nervous system (CNS) infections are among the most devastating infectious diseases in the world with substantially high morbidity and mortality. Bacteria, fungi, viruses, and parasites can invade the CNS. Among bacteria, *Staphylococcus aureus* represents both a human pathogen and a commensal bacterium with approximately 30% of the human population colonized. *S. aureus* bacteremia leads to 1–9% of all bacterial meningitis cases in adults. Moreover, *S. aureus* is the most identified pathogen in brain abscesses with an incidence of approximately 30%. To uncover both bacterial and host factors that determine the outcome of infection, an animal model of systemic staphylococcal infection was utilized for the development of brain abscesses followed by multimodal imaging. This methodology included the use of optical microscopy and matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS). Staphylococcal abscesses developed in 10% of mice infected for six to ten days either in cortical or subcortical brain areas. Focal meningoencephalitis showed communities of bacteria in direct interaction with innate immune cells. Additionally, MALDI IMS revealed notable changes in the abundance of fatty acids such as arachidonic, oleic, palmitic, stearic, and most particularly linoleic acid metabolism, as well as in gangliosides. These changes were visible in both infected and uninfected tissues, highlighting the complex molecular response of the brain to infection. These studies, focused on molecular determinants at the host-pathogen interface during CNS infection, can shed light on future development for novel antimicrobials and may a profound impact on the fields of neurology and neuro-infection biology.

LT7- see also PT22

Targeting Biofilm Structure and Integrity with Free and Microparticle-Based Allicin

Presenter name: **Nina Nováková**

Co-authors: Dominik Maršík, Lucie Mašková, Olga Maťátková, Ondřej Kašpar, Viola Tokárová

University of Chemistry and Technology, Prague, Czech Republic

Bacterial biofilms use quorum sensing as a communication mechanism responsible for production of their own matrix of hydrated extracellular polymeric substances, growth, and resistance to microbial agents. Disruption of these signalling pathways represents a potential strategy for weakening biofilm communities, including the clinically significant pathogen *Pseudomonas aeruginosa*, which poses a major threat especially to patients with cystic fibrosis. Allicin, a reactive compound formed upon garlic tissue disruption, exhibits antibacterial activity and the ability to interfere with quorum sensing. However, its application is limited due to its high chemical instability. In this work, we compare the effects of free allicin produced via enzymatic conversion of alliin by alliinase with allicin generated in situ from spray-dried microparticles. These particles contain separately encapsulated alliin and alliinase, allowing localized formation of the active compound at the target site. The aim is to evaluate differences in antimicrobial activity between the two systems and to assess their potential to enhance the efficacy of selected antibiotics. The study involves inhibition of planktonic *P. aeruginosa*, eradication of static biofilms, and combinatory testing with antibiotics such as polymyxin B, a so-called last-resort antibiotic. Preliminary results indicate possible synergistic effects, although system efficacy depends on the release parameters and the nature of the target environment. Further experiments are required to optimize the formulation and confirm its potential under biologically relevant conditions.

LT8 - see also PT25

Engineering Biosimilar Mucus: Integrating Composition, Rheology and Bioprinting for In Vitro Barrier Model

Presenter name: **Suada Đukaj[1]**

Co-authors: Denisa Lizonová[1], Josef Beránek[2], František Štěpánek[1]

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This project aims to artificially recreate the intestinal mucus barrier with enhanced biosimilarity. In the past symposiums it was shown that we have extracted our own mucins with better rheology and functional properties compared to the commercial ones. We use these mucins in combination with other components that are present in mucus such as lipids and other proteins to fine-tune the artificial mucus rheology. The rheology is a work in progress and is being measured by the TA Instruments rheometer with plate-to-plate geometry where viscosity, storage and loss modulus are being assessed. In parallel, we attempted to achieve physiological mucus thickness in trans-well system using a bioprinter. For this, we first used the native mucus as a reference. The native mucus was bio-printed with various parameters (pressure, nozzle speed, nozzle type, infill density and mucus rheology) and the ones that produced a mucus thickness between 250-300 um were further chosen for validation. The trans-well system in combination with bioprinter that gave the correct thickness was then used to measure first permeation data of Caffeine and Lucifer yellow. These two components were chosen as our compounds for validating the system and were then compared to the HT29-MTX cell model. The permeability of such components will further help us to validate the artificial mucus layer which we hope to use in the future as a predictive tool for drug diffusion studies.

LT13 - see also PT37

Steroids as potential adjuvants: effect on efflux systems and gene expression in *Staphylococcus aureus*

Presenter name: **Jan Špaček**[a]

Co-authors: Brdová Daniela[a], Křížkovská Bára[a], Lipov Jan[a], Kudová Eva[b], Viktorová Jitka[a]

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Steroid substances, including neurosteroids, are known for their role in regulating immune function and defense against pathogens in the human body. In addition to these physiological effects, their potential impact on micro-organisms is increasingly being discussed, in particular through their influence on efflux systems, which are important for the development of antibiotic resistance. In this study, we performed a systematic structure-activity relationship (SAR) analysis of a selected set of endogenous and synthetic steroids, leading to the identification of pregnanolone as a suitable molecule for derivatization. Among the tested compounds, special attention was paid to the compound VCHT-069, which showed enhanced efficacy against resistant strains of Staphylococcus aureus in association with antibiotics. The interaction with efflux pumps was evaluated using the ethidium bromide accumulation test, with results suggesting an effect on the function of these systems. Whole transcriptome analysis further revealed that the test steroid independently affects the expression of genes associated with virulence and stress response. In addition, in combination with erythromycin, there is a significant attenuation of ribosomal gene expression, which probably explains the observed additive effect. Our findings support the possibility of using steroid derivatives as adjuvants that can make antibiotic therapy more effective by targeting bacterial defence mechanisms.

LT14 - see also PT44

ALFI: A Global Database of Alien Fungal Introductions

Presenter name: **Vasilii Shapkin[1]**

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Alien species represent a major threat to global biodiversity. However, unlike macroorganisms, alien fungal introductions remain poorly understood and largely overlooked. This is primarily due to their cryptic lifestyles, which make them difficult to observe and describe using traditional morphological methods. Over the past two decades, fungal species concepts have shifted toward molecular-based classification, with the nuclear ribosomal internal transcribed spacer (ITS) region established as the universal DNA barcode for fungi. The advent of high-throughput sequencing (HTS) has further advanced the field by enabling ITS-based detection of multiple fungal species in a single environmental sample, greatly enhancing our ability to study fungal ecology and biogeography. GlobalFungi, the largest database of fungal HTS data, contains nearly five billion ITS-based observations from over 80,000 geolocated samples. Leveraging this vast dataset with documented records of alien fungal occurrences, opens a unique opportunity to make first steps towards better understanding of alien fungal introductions on a global scale. To address this, we present ALFI (Alien Fungi Introductions) - a global database compiling over 5,000 first records of alien fungal occurrences from various literature sources. ALFI includes data on more than 1,500 fungal species, 200 detection locations, and their reported native geographic ranges. By integrating ALFI with data from GlobalFungi, we analyzed the global distribution patterns of over 500 fungal species, assessed their alien status through phylogeographic analyses, and identified major geographic regions that act as sources and recipients of these introductions. Beyond mapping origins and destinations, we explored factors contributing to successful introductions, including fungal traits and ecosystem characteristics. Building on existing resources, ALFI represents the most comprehensive database of global alien fungal introductions to date, providing a robust, data-driven foundation for advancing research in fungal biogeography.

LT9

Microbiome and Metabolic Disruption in Acute vs. Severe and Enduring Anorexia Nervosa

Presenter name: **Petra Prochazkova**

Co-authors: Janet Jezkova[1,2], Radka Roubalova[1], Katerina Zadakova[1] Kristyna Coufalova[1], Gabriela Kubisova[1], Jakub Kreisinger[3], Jaroslav Semerad[1,4], Alena Nehasilova[1], Tomas Cajthaml[1,4], Helena Tlaskalova-Hogenova[1], Petra Holanova[5], Alena Lambertova[5], and Hana Papezova[5]

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Anorexia nervosa (AN), a serious eating disorder, is associated with marked changes in the microbiome and metabolic profile of those affected. However, direct comparisons of microbiome profiles between individuals with acute AN and those with severe and enduring anorexia nervosa (SEAN) are limited or lacking entirely.

In this study, we investigated gut microbiota diversity and composition in female patients with acute AN or SEAN, and in healthy female controls. We detected group differences across several parameters, including eating behavior, depressive symptoms, experiences of adult stress, and antidepressant usage. Notably, the SEAN group exhibited elevated biomarkers of gut barrier damage and the highest degree of individual variability in gut microbiota composition.

Across both patient groups, several bacterial taxa—including *Faecalibacterium*, *Fusicatenbacter*, *Lachnospiraceae*, and *CAG-56*—were significantly reduced in abundance, while *Erysipelatoclostridium* and *UBA1819* were elevated. Functional predictions based on microbial composition indicated changes in amino acid metabolism and oxidative stress responses, especially in the SEAN cohort.

Both patient groups showed lower serum and fecal levels of GABA, potentially linked to increased abundance of microbial taxa such as *Christensenellaceae*, *Ruminococcaceae*, and *Escherichia-Shigella*, which may negatively influence GABA metabolism. The *Christensenellaceae* family may impair microbial fermentation processes, contributing to altered concentrations of short-chain fatty acids observed in both stool and serum AN samples.

Overall, these findings suggest that chronic form of AN is associated with distinct and potentially more severe alterations in the gut microbiome. A better understanding of microbiome changes over the course of AN may inform future strategies for prevention and treatment.

LT10

Microbiota-Driven Effects on the Development of a Mouse Model of Anorexia Nervosa

Presenter name: **Radka Roubalová**

Co-authors: Petra Procházková[1], Janet Ježková[1,2], Kateřina Zadáková[1], Kristýna Coufalová[1], Tomáš Hrnčíř[1], Tommaso Stephani[1], Jakub Kreisinger[3], Jaroslav Semerád[1], Hana Papežová[4]

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The gut microbiota influences not only physiology, but also behaviour and mental health. Our research has long focused on the influence of the gut microbiota on the development and progression of anorexia nervosa, an eating disorder that significantly affects quality of life.
We transplanted the gut microbiota from acute and chronic patients with anorexia nervosa (AN) and from healthy controls to conventional and germ-free mice. We investigated the development of activity-based anorexia (mouse model of AN) in these mice. First, we determined the differences in the core symptoms of this disease – body weight, food intake and activity of the mice. Mice transplanted with microbiota from acute AN patients consumed significantly less food than mice transplanted with the microbiota from controls and chronic patients. In contrast, mice transplanted with the microbiota from chronic patients had higher running activity than mice transplanted with the microbiota from controls or acute AN patients.
Further, we investigated the effects of the microbiota on appetite-regulating metabolic pathways. In mice transplanted with the microbiota from patients with acute AN, we observed significantly reduced expression of key central hunger-signalling peptides—neuropeptide Y (NPY) and agouti-related peptide (AgRP) corresponding with their lower food intake. In addition, the expression of GABA receptors was altered in these mice. These findings suggest a microbiota-driven effect on hypothalamic AgRP/NPY GABAergic neurons.
Overall, the gut microbiota affects the development of a mouse model of anorexia nervosa. In acute patients, the microbiota appears to influence the central regulation of appetite. In chronic patients, it seems to be more associated with the hyperactivity frequently observed in these patients.

LT11

Ocrelizumab causes transient changes in the composition of intestinal bacteria and modulates the immune response depending on the response to treatment

Presenter name: **Stepan Coufal**[1]

Co-authors: Zuzana Jiraskova Zakostelska[1], Tomas Thon[1], Radka Roubalova[1], Dominika Kadleckova[2], Martina Salakova[2], Ruth Tachezy[2], Tomas Hrncir[3], Miloslav Kverka[1], Veronika Ticha[4], Miluse Pavelcova[4], Pavlina Kleinova[4], Jana Lizrova Preiningerova[4], Ivana Kovarova[4], Jakub Kreisinger[5], Helena Tlaskalova-Hogenova[1] and Eva Kubala Havrdova[4]

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Multiple sclerosis (MS) is an autoimmune disease that leads to the loss of myelin and atrophy of the central nervous system. The role of gut microbiota dysbiosis has been implicated in MS pathogenesis and may also influence treatment outcomes.
In our study, we included 25 newly diagnosed persons with MS (PwMS) with clinically isolated syndrome (CIS), which were treatment-naïve and 9 PwMS on IFN-β who had relapsed and were indicated for treatment with ocrelizumab. The eighty-one healthy control subjects were also recruited. Stool and serum in study groups were collected before the treatment and every 3 months for a minimum of 12 months.
We identified changes in the gut microbiota that are already present in CIS persons who are naive to MS treatment. While treatment responders showed an increasing trend in alpha diversity after 12 months to the point that they approached values similar in healthy controls, non-responders showed a significant decrease in gut microbiota alpha diversity. The main changes were seen in bacteria, including Parabacteroides spp., which are recognized or putative producers of short-chain fatty acids, which support gut barrier functions and have anti-inflammatory potential. We found altered levels of gut barrier biomarkers and antibodies against ten common gut commensal bacteria in the sera of PwMS compared to healthy controls, we also found that this biomarker profile in PwMS was altered by anti-CD20 treatment. Additionally, we found significant decreases in lipopolysaccharide-binding protein and mannose-binding lectin but only in sera of responders during treatment; no changes occurred in no n-responders.
These results suggest a link between intestinal barrier damage and subsequent immune responses associated with microbial translocation and MS pathogenesis and treatment.

LT12

Lysate of *Parabacteroides distasonis* prevents severe forms of experimental autoimmune encephalomyelitis by modulating the priming of T cell response

Presenter name: **Zuzana Jiraskova Zakostelska**

Co-authors: Stepan Coufal[1], Michal Kraus[1], Tomas Thon[1], Petra Prochazkova[1], Zaneta Slavickova[1], Tomas Hrncir[2], Klara Kostovcikova[1], Miloslav Kverka[1], Veronika Ticha[3], Miluse Pavelcova[3], Pavlina Kleinova[3], Jana Lizrova Preiningerova[3], Ivana Kovarova[3], Jakub Kreisinger[4], Eva Kubala Havrdova[3], Helena Tlaskalova-Hogenova[1], Miloslav Kverka[1]

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The gut microbiota influences the reactivity of the immune system, and *Parabacteroides distasonis* has emerged as an anti-inflammatory commensal. Here, we investigated whether its lysate could prevent severe forms of neuroinflammation in experimental autoimmune encephalomyelitis (EAE) in mice and how this preventive strategy affects the gut microbiota and immune response. Lysate of anaerobically cultured *P. distasonis* (Pd lysate) was orally administered to C57BL/6 mice in four weekly doses. One week later, EAE was induced and disease severity was assessed three weeks after induction. Fecal microbiota changes in both vehicle- and Pd lysate-treated animals was analyzed by sequencing and qPCR, antimicrobial peptide expression in the intestinal mucosa was measured by qPCR, and immune cell composition in the lymph nodes was measured by flow cytometry. Pd lysate significantly delayed the development of EAE and reduced its severity when administered prior to disease induction. EAE induction was the main factor in altering the gut microbiota, decreasing the abundance of lactobacilli and segmented filamentous bacteria. Pd lysate significantly increased the intestinal abundance of the genera *Anaerostipes*, *Parabacteroides* and *Prevotella*, and altered the expression of antimicrobial peptides in the intestinal mucosa. It significantly increased the frequency of regulatory T cells, induced an anti-inflammatory milieu in mesenteric lymph nodes, and reduced the activation of T cells at the priming site. Pd lysate prevents severe forms of EAE by triggering a T regulatory response and modulating T cell priming to autoantigens. Pd lysate could thus be future modulator of neuroinflammation that increases the resistance to multiple sclerosis.

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PT3

Lipocalin-enterobactin complexes revealed by native mass spectrometry and molecular modelling

Presenter name: **Summra Ahmed**

Co-authors: Summra Ahmed[a,b], Hynek Mácha[a], Josef Chmelík[a], Dominika Luptáková[a], Alan Kádek[d], Milan Raška[c], Vladimir Havlíček[a,b]

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One of the key factors in developing an infection is competing for nutrients. Some bacteria synthesize the enterobactin (Ent) siderophore, which chelates iron from a mammalian host. In response, the host secretes lipocalins (Lcn) to impede the growth of bacterial siderophore populations at the infection site. We investigated the intercalation of Ent into Lcn, including lipocalin-1 (Lcn1) and lipocalin-2 (Lcn2), pockets using native mass spectrometry and the computational tools SwissDock, KVFinder, LigPlot, and Chimera. Non-covalent complexes [Lcn1-Ent] and [Lcn2-Ent] were recorded using electrospray ionization (ESI) quadrupole time-of-flight mass spectrometry (Synapt G2Si, Waters), with homemade gold-plated ESI emitters. The stability of the [Lcn-Ent] complexes were tested within the following ranges: pH (5.4–9), Ent concentration (0.2–200 µM), and ion block temperature (50–120 °C). Optimal complex formation conditions were found to be a Lcn:Ent ratio of 1:1 at a pH of 7.4 and a concentration of 20 µM. Temperature had little effect. Partial or complete intercalation of Ent into Lcn1 or Lcn2, respectively, was recorded with the amino acids Lys114, Asp25, and Leu33 in [Lcn1-Ent] and Lys125 and Lys134 in [Lcn2-Ent], which are critical to complex formation. The physicochemical parameters of the noncovalent complexes, when recorded in vitro and in silico, may provide critical new insights into the immune responses elicited by the mammalian host.

For PT4 abstract see LT3

PT5

Biosynthesis, biodegradation and determination of the non-protein amino acid β-cyano-L-alanine, an intermediate of cyanide detoxification in plants

Presenter name: **Barbora Křístková[1,2]**
Co-authors: Lenka Rucká[3], Romana Příhodová[1], Natalia Kulík[4], Vladimír Křen[1], Pavla Bojarová[1,5], Ludmila Martínková[1]

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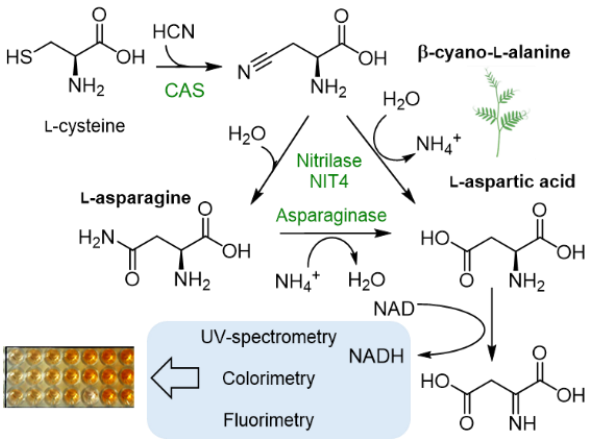
β-Cyano-L-alanine (cyanoalanine) and its dipeptide belong to the antinutritive compounds of legumes. Cyanoalanine and its dipeptide (neurotoxins) are typical of vetch (*Vicia sativa*), an important forage plant. Therefore, vetch must be used with caution. In addition, vetch is difficult to distinguish from a type of lentil. This has been misused: The cheap vetch seeds have been illegally added to lentils.
Cyanoalanine is formed during the detoxification of HCN in plants. HCN is mainly formed during the biosynthesis of the plant hormone ethylene. Cyanoalanine is synthesized from L-cysteine and HCN under the control of β-cyano-L-alanine synthase (CAS; EC 4.4.1.9). Here, we investigated the CAS of *Spinacea oleacea* (spinach) and overproduced its mature form in *Escherichia coli*. The enzymes that detoxify cyanoalanine to asparagine and aspartic acid (nitrilases NIT4 in plants and fungi) were also investigated by us.
We then focused on the use of CAS and NIT4 in chemical analysis (Scheme 1). The enzymes are promising for the determination of cyanide, whereby the aspartic acid produced by NIT4 is dehydrogenated by aspartate dehydrogenase and NADH is quantified. Parts of the cascade can be used for the determination of cyanoalanine or aspartic acid. In addition, asparagine (precursor of the toxic acrylamide) can be determined after addition of asparaginase. These reactions are promising for use in sensors and kits for quality and safety control of food and feed.



Scheme 1. Biosynthesis and biodegradation of β-cyano-L-alanine and the use of the enzymes for analytical methods

PT6

Prenylation and Geranylation of Flavonoids for Improved Bioavailability and Anti-Inflammatory Effects

Presenter name: **Alice Pomeislová**

Co-authors: Martina Hurtová, Lucie Petrásková, Kateřina Valentová

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Flavonoids are secondary plant metabolites that play an important role in human diet. It was found that flavonoids may have beneficial effects on cardiovascular health and exhibit anti-inflammatory and antioxidant properties. Structural modifications such as prenylation (C_5-alkyl chain introduction) and geranylation (C_{10}-alkylation) strongly affect physicochemical properties and biological activity of flavonoids. Existing studies have shown that these lipophilic side chains increase membrane permeability and slow down the biodegradation. Moreover, prenylated flavonoids have demonstrated anti-inflammatory potential by modulating the NF-κB pathway, which leads to inhibition of COX and LOX enzymes.

The increased bioactivity of these derivatives may also be influenced by their interactions with the gut microbiota. Although the exact mechanism remains unclear, it is hypothesized that prenylation and geranylation could affect microbial degradation or biotransformation of flavonoids, which may alter their pharmacokinetics and biological effects. However, these assumptions require systematic experimental validation.

In this study, C8-prenylation of selected flavonoids (three flavones and one flavanone) was achieved via a selective palladium-catalyzed C7-O-reverse-prenylation, followed by a Claisen rearrangement to relocate the prenyl group to the C8 position. Using this method, 8-prenylquercetin, 8-prenylmorin, 8-prenyl-2,3-dehydrosilybin, and 8-isoquercitrin were synthesized. This synthetic strategy was also applied to the preparation of 8-geranylated quercetin.

All the obtained compounds were fully structurally characterized (NMR, MS) and will be subjected to *in vivo* pharmacokinetic evaluation in mice and fermentation studies with human faecal inoculum to determine the microbial metabolism. Also, *in vivo* anti-inflammatory assays will be performed.

For PT7 abstract see LT4

For PT8 abstract see LT5

PT9

Linking c-di-AMP homeostasis and cell division regulation in *Streptococcus pneumoniae*

Presenter name: **Tomáš Beneš[1,2]**

Co-authors: Karolína Buriánková[1], Aleš Ulrych[1], Pavel Branny[1], Zdeněk Kameník[1], Tomáš Vomastek[1]

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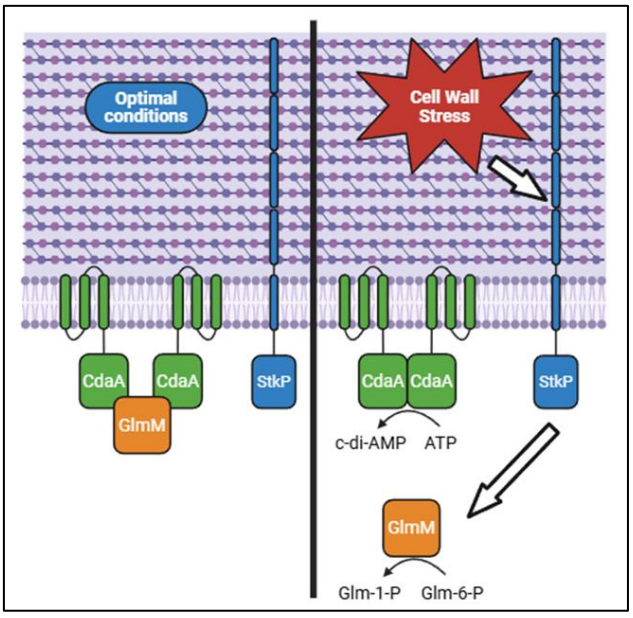
Secondary messengers provide a rapid response to changes in the environment. One of the bacterial secondary messengers not found in eukaryotes is c-di-AMP. This compound regulates the potassium level in the cell and thus the osmotic pressure. However, it is also associated with biofilm formation or sporulation. C-di-AMP is synthesised by a single diadenylate cyclase CdaA in *Streptococcus pneumoniae*. This enzyme is encoded in a highly conserved operon together with the phosphoglucosamine mutase GlmM, which produces glucosamine-1-phosphate, an important precursor for cell wall biosynthesis. It has been described that GlmM interacts with CdaA and inhibits the production of c-di-AMP. However, the exact mechanism of how GlmM regulates the level of c-di-AMP in the cell is not known. Our previous studies have shown that GlmM is phosphorylated by the serine/threonine kinase StkP, which is involved in the regulation of cell division. Based on this connection, we hypothesise that there is a link between StkP and the c-di-AMP metabolism. Our preliminary results indicate that CdaA interacts directly with StkP and that CdaA is localised in the midcell. However, CdaA is most likely not a substrate of StkP as we could not detect any phosphorylation of CdaA *in vivo*. Thus, the interplay between these proteins probably occurs indirectly with the involvement of GlmM. The level of c-di-AMP is higher when CdaA is co-expressed in *E. coli* with StkP and GlmM than when only CdaA and GlmM are present. In conclusion, these results indicate that StkP is involved in the homeostasis of c-di-AMP.



Graphical representation of the possible regulation of c-di-AMP synthesis by the kinase StkP. Created in Biorender.

PT10

EUROTOXIC ACTIVITY OF *BORDETELLA* DERMONECROTIC TOXIN AT SUB-PICOMOLAR CONCENTRATIONS

Presenter name: **Ondřej Staněk[1]**

Co-authors: IRENA LINHARTOVA [1], TOMÁŠ VOMASTEK[1], JANA HOLUBOVÁ [1], ZUZANA NICHTOVA [2], PETR MACEK [2], MILOSLAV KORINEK [3], TEREZA SMEJKALOVA [3], PETER ŠEBO [*,1]

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Lysing pathogenic *Bordetella* bacteria release a neurotropic dermonecrotic toxin (DNT), which is endocytosed into host cells and permanently activates RhoA family GTPases by polyamination or deamidation of glutamine residues in their switch II regions (e.g., Gln63 of RhoA). In *B. bronchiseptica*, DNT facilitates bacterial colonization in the nasal cavity of pigs and inhibits the differentiation of nasal turbinate bone osteoblasts, contributing to atrophic rhinitis. However, the role of DNT in virulence of *B. pertussis* and pathogenesis of whooping cough remains unclear. Recent studies identified T-type voltage-gated calcium channels (Cav3.1 and Cav3.2) as receptors for DNT. Our findings confirm that DNT interacts with these channels, facilitating calcium entry into cells and enhancing its RhoA polyaminase and deamidase activity. However, we did not observe a direct binding of DNT to cells via these channels, suggesting that additional receptor(s) may be involved. We developed a method to purify large quantities of lipopolysaccharide (LPS)-free recombinant DNT with high biological activity on sensitive cells, including its fragments and detoxified variants. Contrary to earlier reports, we show that the C-terminal enzymatically active domain specifically binds to sensitive cells, while the N-terminal "binding" domain does not (data not shown). Our results reveal that even extremely low concentrations of DNT (femtomolar) disrupt the function of primary rat neurons cultured *in vitro.* DNT damages astrocyte protrusions, halting their support for neurons, leading to progressive neuronal death and loss of action potential transmission. Additionally, intravenous administration of as little as 3 ng (18 fmol) of DNT in mice causes weight loss and severe neurological symptoms, ultimately resulting in death. We report significant progress in understanding of the molecular mechanisms underlying DNT's effects at such low concentrations and mapping of its cell-binding domains, shedding light on its potential role in *B. pertussis* pathogenesis.

PT11

Bordetella pertussis Toxins Drive the Emergence of a Unique CD8$^+$ T Cell Subset in the Respiratory Tract

Presenter name: **Jana Holubová[2]**

Co-authors: Anna Kratochvílová[1], Ondrej Stanek[2], Peter Sebo[2], Ondrej Stepanek[1]

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Bordetella pertussis, the causative agent of whooping cough, is known for its ability to manipulate the host immune defence through the action of its enzymatically active adenylate cyclase, dermonecrotic and pertussis toxins (*e.g.* ACT, DNT and PT). The impact of their action on T cell populations and airway-specific mucosal immunity, however, remains poorly understood. We thus investigated the nasal cavity colonization dynamics of wild-type and toxin mutant strains of *B. pertussis* in a mouse model, focusing on the characterization of T cells in the mucosa of the upper respiratory tract (URT) and in lungs.

Our findings identified a distinct subset of CD8$^+$ T cells with an atypical phenotype that emerged both in the URT and lungs following *B. pertussis* infection. These cells exhibited an unconventional phenotype, marked by the expression of the transcription factor Eomes and checkpoint-inhibition receptors (Tigit and PD-1). Pertussis toxin (PT) activity was strongly implicated in driving this phenotype, while the effects of DNT and ACT action on T cell populations were more moderate. Additionally, we observed reduced T cell infiltration in the nose and lungs after infection with the mutant strain producing an enzymatically inactive PT$^-$toxoid.

The emergence of the "strange" CD8$^+$ T cells following infection with PT$^+$ wild-type bacteria reveals a novel immunomodulatory mechanism by which *B. pertussis* toxin action impacts host adaptive immunity. Further studies will focus on the function and antigen specificity of these atypical CD8$^+$ T cells to elucidate their role in the immune response and disease pathology.

PT12

Extreme C-terminus of the FhaB prodomain is essential for interaction of *Bordetella pertussis* with nasal ciliated epithelial cells

Presenter name: **Ladislav Bumba**

Co-authors: David Jurnecka, Josef Chmelik, Jana Holubova, Ondrej Stanek, Peter Sebo,

Institute of Microbiology of the CAS, Prague 4, Czech Republic

Filamentous hemagglutinin (FHA), a major virulence factor of classical *Bordetella* species, is a rod-shaped molecule that plays a crucial role in bacterial adherence to ciliated epithelial cells of the upper respiratory tract while modulating the host innate and adaptive immune responses. FHA is translated as a 360-kDa FhaB precursor, which is exported across the outer bacterial membrane by a two-partner secretion mechanism involving the outer membrane protein FhaC. After secretion, FhaB undergoes processing by the surface-exposed SphB1 protease, releasing an N-terminal 'mature' 220-kDa FHA protein into the external environment. The remaining C-terminal 130-kDa FhaB prodomain is thought to regulate the maturation process and is rapidly degraded in the periplasm. Here, we demonstrate that the extreme C terminus (ECT) of the FhaB prodomain plays a pivotal role in *B. pertussis* virulence. NMR-based structural analysis of ECT, which consists of the highly conserved C-terminal 100 residues of FhaB, revealed that it adopts a rigid, 'pilin-like' protein fold. Deletion of the ECT sequence (ΔECT) resulted in a significant reduction in bacterial colonization of the nasal cavity in infected mice, comparable to the colonization defect observed in a *B. pertussis* strain lacking *fhaB* (ΔFhaB). Strikingly, the ΔECT strain completely lost its ability to bind cilia on human nasal epithelial cells cultured at the air-liquid interface. These findings provide novel insights into FhaB biology and underscore the indispensable role of ECT in *Bordetella* adherence to ciliated epithelial cells in the upper respiratory tract.

PT13

Deciphering the early innate immune response of nasal mucosa to *Bordetella pertussis* infection

Presenter name: **Ludmila Blechová (Brázdilová)[1,2]**

Co-authors: Jana Holubová[1], Ondřej Staněk[1], Veronika Niederlová [3], Aleš Neuwirth[3], Juraj Michálik[3], Anna Kratochvílová[3], Ondřej Štěpánek[3], Ladislav Bumba[1], Peter Šebo[1]

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3 *Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic*

Bordetella pertussis is a strictly human pathogen that elicits a highly contagious respiratory illness known as pertussis, or whooping cough. Current mouse models enabled identification of many bacterial virulence factors and development of pertussis vaccines, but the mechanisms underlying the process of *B. pertussis* transmission during the catarrhal phase of pertussis disease remain largely unexplored due to lack of a convenient animal model. Recently, we have used immunodeficient MyD88 knock-out mice to achieve a human-like high level of nasal mucosa infection, which triggered rhinitis and catarrhal shedding of bacteria from mouse nasal cavity and transmission of the infection onto co-housed adult animals (Holubova et al., 2022, PLoSPathog 18: e1010402). Here, we compared the early innate immune response of the conventional C57BL/6 mice and the MyD88 knock-out mice upon intranasal challenge with *B. pertussis*. Flow cytometry analysis of cells from nasal tissue shows how multiple immune cell populations infiltrate the nasal mucosa after infection in both strains of mice. Single-cell RNA sequencing revealed that nasal mucosa response of conventional mice infected with *B. pertussis* is characterized by the expansion of a highly activated neutrophil subset characterized by an interferon-stimulated gene signature, which does not develop in the MyD88KO mice. A similar interferon-stimulated gene signature is also observed in the lymphoid compartment and epithelial cells. Moreover, *B. pertussis* infection is associated with an early upregulation of expression of genes encoding antimicrobial peptides (for example Lipocalin 2) and chemoattractant molecules (for example Interleukins 1b, 12 and 18, Lix and Cxcl10), which is severely delayed in MyD88 knock-out mice. These results open the way for a detailed understanding of innate immune responses involved in *B. pertussis* clearance from nasal mucosa of the host and enable better study of early transmission between hosts.

PT14

The mystery of the *Bordetella* type III secretion system effector protein BteA

Presenter name: **Tania Romero Allsop**

Co-authors: Eliska Sedlackova[1], Ivana Malcova[1], Ladislav Bumba[2], Jana Kamanova[1]

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Bordetella pertussis is the causative agent of whooping cough in humans, a respiratory disease that has recently experienced a resurgence. In contrast, *Bordetella bronchiseptica* infects the respiratory tract of various mammalian species, causing symptoms ranging from asymptomatic chronic carriage to acute illness. Both pathogens utilize type III secretion system (T3SS) to deliver the effector protein BteA into host cells. BteA is a 69 kDa protein composed of an N-terminal lipid raft targeting (LRT) domain and a C-terminal cytotoxic domain of approximately 526 amino acids. The AlphaFold 3 model of BteA shows low confidence in the cytotoxic domain and reveals no structural similarity to known proteins. Once inside host cells, BteA induces rapid, caspase-1-independent necrosis through a poorly understood mechanism.
Here, we show that BteA triggers a rapid and sustained influx of calcium ions, which is associated with plasma membrane permeabilization. Using a yeast heterologous expression model, we demonstrate that the N-terminal region (residues 1-312) interacts with the C-terminal region and can recruit it to the membrane. These interactions are supported by crosslinking experiments that detect interactions between putative helix 9 and helix 11. Small-angle X-ray scattering (SAXS) analysis confirms that BteA behaves as a monomer and fits the AlphaFold 3 model well ($\chi^2 = 1.6$). Hydrogen-deuterium exchange mass spectrometry (HDX-MS) reveals both structured and intrinsically disordered regions. Cryo-electron microscopy 2D class averages further support the overall shape of BteA, though currently at low resolution. In the future, we will focus on nanobody stabilization to enhance structural resolution.

PT15

Bimodal Behaviour of Intracellular *Salmonella*

Presenter name: **Milada Kambová[1]**

Co-authors: Alona Dreus[1], Paulina Mathéová[1], Jana Schmidtová[1], Barbora Pravdová[1], Michaela Blažíková[2], Martin Čapek[2,3], Ondřej Černý[1]

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abstract confidential

PT16

Cyclic di-GMP signaling and intracellular adaptation of *Aeromonas veronii* in free-living amoebae

Presenter name: **Jan Blumenstein**

Co-authors: Denisa Vondrova, Sofia Spagnolo, Hana Michova, Jana Kamanova

Laboratory of Infection Biology, Institute of Microbiology, Czech Academy of Sciences, Videnska 1083, Prague 142 00, Czech Republic

Aeromonas veronii is a ubiquitous environmental bacterium that causes disease in fish and is increasingly recognized as an emerging human pathogen. Its persistence in aquatic environments may be supported by free-living amoebae, such as *Acanthamoeba castellanii*, which serve as natural reservoirs for bacterial pathogens by providing a protective intracellular niche. Our work investigates the molecular mechanisms underlying the interaction between *A. veronii* and amoebae, with a focus on cyclic di-GMP (c-di-GMP) signaling in intracellular adaptation.
We demonstrate that *A. veronii* survives phagocytosis by *A. castellanii* and is capable of intracellular replication. To identify the genetic determinants required for this adaptation, we are constructing a high-density transposon mutant library in *A. veronii* and will perform transposon insertion sequencing (Tn-seq) following amoebal infection. This genome-wide screen will identify genes involved in intracellular survival and reveal novel regulatory pathways.
In parallel, we are implementing a biosensor to measure intracellular c-di-GMP levels under various conditions, including low pH environment that mimic the phagosomal compartment. Preliminary results indicate that c-di-GMP levels increase under acidic conditions, supporting its role as a key regulator of bacterial behavior, including intracellular adaptation.
Our results aim to improve understanding of mechanisms underlying bacterial persistence within protozoan hosts.

For PT17 abstract see LT6

PT18

Facing the Challenges of Untargeted Metabolomics: Our Path to Workflow Development and Data Integration

Presenter name: **Tommaso Stefani**[a]

Co-authors: Anna Michl[b], Ondřej Hřebíček[a], Martin Schwarzer[b], Zdenek Kamenik[a]

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Untargeted metabolomics requires robust workflows that ensure data quality while handling the complexity of large datasets generated by advanced LC-MS instrumentation. Over the past year, we have developed a comprehensive workflow encompassing chromatographic and mass spectrometry method development, system suitability and quality control (QC) strategies, and multi-platform data processing, combining commercial and open-source metabolomics tools.

We present a modular data processing workflow for untargeted metabolomics that integrates tools across commercial and open-source platforms. Peak picking and feature table assembly are performed using either Bruker's MetaboScape or MZmine, followed by statistical analysis in MetaboAnalyst. Metabolite annotation combines Bruker's METLIN-based MetaboBase, our in-house spectral library, and the community-curated GNPS resource. In silico annotations are generated via SIRIUS, and molecular networking with metadata mapping is constructed using GNPS. All results are consolidated into a single network visualized in Cytoscape. The workflow's modular design and integration across platforms set the foundation for future automation, aiming to streamline data processing and enhance reproducibility.

PT19

Development of Software for LC-MS Data Processing of Oligonucleotides

Presenter name: **Evgeniya Biryukova**[1,2]

Co-authors: Marek Polák[1], Petr Novák[1]

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Ongoing improvements in LC-MS data processing tools are crucial for advancing nucleic acid research and therapeutic innovation. We present a Python-based software tool designed for the efficient analysis and interpretation of oligonucleotide fragmentation patterns. Initially developed for the study of oxidative cleavage induced by FPOP (Fast Photochemical Oxidation of Proteins), the software generates theoretical libraries of monoisotopic fragment masses across multiple charge states and matches them to experimentally detected peaks within a defined mass error tolerance. The newly expanded functionality goes beyond radical-induced fragmentation. The tool now supports simulation of enzymatic cleavage, including products generated by RNases T1 and U2 acting on RNA, and performs automatic annotation of the corresponding fragments. Additional features include graphical output and fragment quantification, enabling a flexible and efficient workflow.
In future development phases, the software could be applied to miRNA analysis from body fluids to support early diagnostics of inflammatory or cancer-related diseases. The tool supports both qualitative and quantitative analysis and, thanks to its intuitive graphical user interface, is accessible even to users with minimal programming experience.
The software was developed within the INTER-MICRO project and serves as an example of effective integration of bioinformatics into modern biomedical research.

PT20

Structural Dynamics of the CyaA Acylated Segment Drive Membrane Invasion: Insights from HDX-MS

Presenter name: **Zuzana Kalaninova[1, 2]**

Co-authors: Petr Man[1], Adriana Osickova[1], Jiri Masin[1], Peter Sebo[1]

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[2] *Faculty of Science, Charles University, Prague 2, Czech Republic*

The adenylate cyclase toxin (CyaA) from Bordetella pertussis is a bifunctional virulence factor essential for early host colonization, exhibiting both adenylate cyclase (cytotoxic) and pore-forming (hemolytic) activities. Proper folding of its acylated segment (AS) is crucial for membrane interaction and functional activity. In this study, we developed a robust hydrogen-deuterium exchange mass spectrometry (HDX-MS) workflow to investigate structural dynamics of full-length CyaA and assess the impact of specific mutations and differential acylation on AS folding.
Wild-type CyaA, mutants (W876F and D880L), and variants with distinct acylation patterns were expressed, purified, and refolded in vitro under calcium-dependent conditions. Optimized proteolytic digestion offered excellent coverage of the acylated region across a range of urea concentrations required for protein solubility. HDX-MS analysis revealed that Trp876 plays a key role in stabilizing acylated loops critical for ß2-integrin-independent cell entry. Its substitution disrupted AS structure and impaired activity on CR3-negative cells. In contrast, the D880L mutation, which alters a newly described calcium-binding site, enhanced structural stability and increased cell binding, translocation efficiency, and hemolytic activity.
Comparative analysis of protoxin (non-acylated), non-canonical acylation (C14), and WT-like C16 acylation variants highlighted the importance of acyl chain composition in modulating AS folding and function. The HDX-MS platform established here enables high-resolution mapping of conformational changes in complex, multidomain bacterial toxins and provides mechanistic insight into virulence regulation, informing future efforts in therapeutic targeting.

PT21

Quantitative Cross-linking Mass Spectrometry Using Data-Independent Acquisition as a Novel Tool for Study of Structural Rearrangement of Proteins in Bacteria

Presenter name: **Michael Karpisek[1,2]**

Co-authors: Valerie Prochazkova[1,2], Michal Rosulek[1,2], Zdenek Kukacka[1] and Petr Novak[1,2]

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Chemical cross-linking in combination with mass spectrometry (CXMS) has been developed into a powerful tool for mapping interaction networks and three-dimensional structures of proteins and their complexes. However, proteins are intrinsically dynamic, and they can form different conformations. Adding quantitative information to CXMS offers a unique opportunity to study flexibility and structural rearrangement of proteins. In this study, we report the benefits of utilizing data-independent acquisition and novel MS-cleavable cross-linker disuccinimidyl dipropionic urea (DSPU).

First, we used different mixtures (9:1, 1:1 and 1:9) of DSPU and its isotopically labeled analogue to modify model proteins testing quantitative potential of our strategy. Subsequently different structural forms of same protein were modified by labeled and non-labeled reagents to quantify the structural rearrangement upon ligand binding. Both, the cross-link formation and quantification were performed in single data-independent experiment where peptides were measured in the broad-band mode with high mass accuracy and subsequently fragmented without isolating precursor ions at fixed collision energy.

Acquired data on studied proteins clearly demonstrate the potential of our quantitative cross-linking strategy. The high mass accuracy in MS enables unambiguous identification of cross-linked peptides and due to the presence of isotopically labelled reporter ions in MS/MS spectra, it is possible to improve the qualitative and quantitative aspects of cross-linking experiments. Observed changes nicely overlap with high resolution structural models and previously published data. Our results lead to an assumption that presented data-independent acquisition method can be utilized for quantitative cross-linking experiments studying structure and dynamics of protein assemblies in solution.

For PT22 abstract see LT7

PT23

Oil Marbles as SMEDDS-Based Carriers for Enhanced Delivery of Curcumin

Presenter name: **Eva Králová**

Co-authors: Adéla Brejchová, Karolína Hintnausová, František Štěpánek

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Curcumin, a natural compound, is frequently studied for its therapeutic potential. However, its poor solubility and low oral bioavailability limit its clinical use. To address these limitations, a self-microemulsifying drug delivery system (SMEDDS) was proposed as a potential solution. SMEDDS, composed of a mixture of oil, surfactant, and co-surfactant, spontaneously form fine oil-in-water emulsions upon contact with aqueous environments. The resulting microemulsion droplets can enhance drug stability, increase absorption, and increase surface area.
In this study, a novel formulation approach was developed using liquid oil marbles—SMEDDS-based core particles coated with powder. These oil marbles combine the bioavailability-enhancing properties of SMEDDS with the physical advantages of solid dosage forms, including improved handling and mechanical stability.
Formulation development involved solubility screening of curcumin in various excipients, supported by pseudo-ternary phase diagrams to identify optimal self-emulsifying compositions. The SMEDDS mixtures were then formulated into oil marbles and coated with hydroxypropyl methylcellulose. These formulations were characterized for droplet size upon dispersion, homogeneity, and mechanical resistance.
Dissolution testing demonstrated significantly improved release kinetics compared to unformulated curcumin extract, confirming the system's capability to enhance oral delivery. This approach presents oil marbles as a potential delivery system for lipophilic drugs, such as curcumin, combining the strengths of both liquid lipid-based systems and solid oral dosage forms.

PT24

Glucan Particles as an Innovative Oral Delivery System for Cannabidiol

Presenter name: **Adéla Brejchová**

Co-authors: Eva Králová, Adam Opravil, Elizaveta Mutylo, Gabriela Ruphuy Chan, František Štěpánek

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Poor aqueous solubility of many active pharmaceutical ingredients, including cannabidiol (CBD), significantly limits their oral bioavailability. One of many promising approaches to address this issue may be the use of glucan particles (GPs) derived from *Saccharomyces cerevisiae* yeast. These hollow, porous shells serve as biocompatible carriers that enhance solubility by maintaining the encapsulated substance in an amorphous state, while also offering potential immunomodulatory benefits.
In this study, GPs were prepared via multistep extraction involving alkaline and acidic treatments, followed by sequential washing with water, isopropanol, and acetone. The resulting powder was lyophilized and used for CBD encapsulation through spray-drying. The suspension, containing ethanol-dissolved CBD and GPs (final GP concentration 20 mg/mL), was processed on a Büchi B-290 Mini Spray Dryer with an ultrasonic nozzle. Various CBD loadings (20–40 wt%) were tested under optimized conditions.
The resulting dry powders were evaluated for encapsulation efficiency, dissolution properties, and suitability for further formulation. Dissolution testing revealed improved CBD release compared to raw material, indicating enhanced solubility. Preliminary results also support the feasibility of compressing GPs into tablet form, offering a patient-friendly dosage option with potential for controlled release.
This study demonstrates the potential of glucan particles as a versatile oral delivery platform for poorly water-soluble compounds such as CBD, bridging the gap between enhanced bioavailability and patient adherence through tablet formulation.

For PT25 abstract see LT8

For PT26 abstract see FT13

PT27

Western Diet Disrupts Anti-Tumor Immunity and Promotes Melanoma Progression Through Myeloid and T Cell Modulation

Presenter name: **Janaina L. S. Donadio**

Co-authors: Anna Pavlicova, Stepan Coufal, Veronika Motuzová, Michal Krauss, Miloslav Kverka

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Melanoma incidence is higher in countries where Western-type diets (WD) are common. While WD is known to promote a tumor-supporting environment, the immune mechanisms behind this — and how they might involve the gut microbiota — remain poorly understood.
In this study, we investigated how WD shapes anti-tumor immunity using a syngeneic melanoma model. Female C57BL/6 mice were fed either WD or control diet (CT) for 15 days, followed by subcutaneous implantation of B16-F10 melanoma cells. We conducted two parallel experiments:
(1) a survival study, where tumor growth and survival were monitored over time; and
(2) an immune profiling study, where tumors, spleens, and mesenteric lymph nodes (mLN) were analyzed by flow cytometry at day 21 post-implantation.
In the survival study, WD-fed mice showed larger tumors and reduced overall survival compared to CT-fed mice. Interestingly, in the follow-up immune profiling study, although tumor sizes were similar between groups, immune cell patterns were different. WD-fed mice had an increased accumulation of monocytic myeloid-derived suppressor cells (MO-MDSCs) and non-antigen-presenting myeloid cells within the tumors, suggesting enhanced local immunosuppression and impaired $CD4^+$ T helper cell activation. In the mLN, T regulatory cells (Tregs, $CD25^-FoxP3^+$) were reduced, indicating disrupted mucosal immune regulation. In the spleen, there was an increase in Th17 cells, pointing to a shift toward inflammatory T cell responses.
These findings highlight that dietary interventions reshape anti-tumor immunity in complex ways: WD appears to drive systemic inflammation while maintaining a less immunogenic tumor. These effects suggest an alteration in gut microbiota composition, known to modulate Th17, Tregs, and myeloid populations. We propose that dietary patterns induce changes in the microbiota and immune system, creating different environments across immune tissues, potentially modulating how the body responds to tumors.

PT28

Oral colistin improves the therapeutic response of tumors to anti-PD1 treatment in an IFN-γ-dependent mechanism

Presenter name: **Miloslav Kverka**

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Gut dysbiosis plays an important role in the response to cancer immunotherapy and antibiotic treatment is associated with a poorer response to immunotherapy. Here, we investigated how colistin (narrow-spectrum antibiotic to G- bacteria) affects the outcome of anti-PD1 cancer therapy. We exposed mice bearing MC-38 tumor and treated with anti-PD1 to colistin. The effect of colistin on the microbiota was analyzed by sequencing, immune cells in the mesenteric lymph nodes and tumors were analyzed by flow cytometry and the significance of specific immune factors was investigated using IFN-γ-deficient mice and IL-17A depleting antibodies.
Colistin enhanced, rather than impaired, the efficacy of anti-PD1 on MC-38 adenocarcinomas. It only slightly altered the composition of the gut microbiota, but it increased the production of IFN-γ in the gut and TME and IL-17A in the TME, and the TME contained a significant NK cell and CD8+ T cell signature on RNAseq. Depletion of IL-17A did not alter the effect of colistin, but the effect was lost in IFN-γ-deficient mice or when cell egress was blocked with FTY720.
Some antibiotics, such as colistin, enhance the anti-PD1 therapeutic effect in MC-38 adenocarcinomas by increasing the antitumor response in the tumor microenvironment. This effect is driven by IFN-γ, but not IL-17A, and requires unimpeded migration of T cells.

PT29

Depletion of Myeloid Cells Reduces Tumorigenesis Driven by a High-Protein Diet in a Colitis-Associated Cancer Model

Presenter name: **Veronika Motúzová**

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Diet shapes the tumor microenvironment in colorectal cancer by modulating the reactivity of the immune system. We investigated the immunomodulatory properties of increased dietary protein intake in the early phase of tumorigenesis in a mouse model of colitis-associated cancer.
BALB/c mice were fed a synthetic control diet (CD) or a high-protein diet (HPD) and subsequently treated with azoxymethane and dextran sodium sulfate to induce colitis-associated cancer. The involvement of macrophages in tumorigenesis was examined by depleting them with clodronate-loaded liposomes. Immunophenotyping was performed by flow cytometry, and cytokine production in Peyer's patches (PPs) was measured by ELISA. Colonoscopic screening was performed to assess tumor characteristics.
HPD-fed mice developed more numerous and larger tumors and had a lower proportion of systemic CD8+ T cells. However, clodronate-mediated depletion of macrophages during early tumorigenesis significantly reduced tumor incidence and restored CD8+ T cells. In the spleens of clodronate-treated mice, a proinflammatory macrophage subpopulation was partially depleted, along with a significant reduction in neutrophils, while NK, NKT and DC cells increased. In addition, HPD-fed mice tended to have elevated proinflammatory TNFα, IL-17, IL-1β and S100A8 in PPs, with TNFα and IL-17 even higher in clodronate-untreated mice.
Our findings demonstrated that HPD contributes to intestinal inflammation and exacerbates tumor development driven either by macrophage or neutrophil stimulation.

This study was supported by the Czech Academy of Sciences (LQ200202105) and the Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.01.01/00/22_008/0004597).

PT30

Immune Response to Microbial Transglutaminase after polyinosinic-polycytidylic acid (Poly I:C) immunization in a Murine Model

Presenter name: **Maliha Rizwan**

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Microbial transglutaminase (mTG) is widely used in the food industry due to its ability to modify proteins to improve food quality. While tissue transglutaminase (tTG) is a known autoantigen in celiac disease (CD). Despite their structural differences, both catalyze the modification of gluten. Here we aim to study the mucosal immune response to mTG administered via oral gavage.

We immunized C57BL/6 mice intraperitoneally with polyinosinic-polycytidylic acid and subsequently challenged orally with mTG from *Streptoverticillium mobaranese* for five days. 24 hours after the last immunization, we analyzed T cell response from the cells isolated from duodenal tissue by multicolor flow cytometry. We also cultured excised Payer's patches from immunized mice to analyze changes in cytokine production.

We detected increased proportions of $CD4^+CD25^+$, $CD4^+CD25^+CD69^+$, $CD8^+CD69^+$, $CD8^+CD44^+$, and γδ TCR^+ T cell populations. Additionally, a significant rise in IFN-γ^+ and TNF-α^+ producing $CD4^+$ T cells was observed in duodenal tissues. Interestingly, immunization revealed elevated CCL2 and IL-17 levels in mice receiving mTG alone, while IFN-γ levels were markedly increased in mice receiving Poly I:C along with mTG. Moreover, all immunized groups showed increased IL-12 and IL-1β, indicating a consistent innate pro-inflammatory response

These findings demonstrate that oral exposure to mTG, particularly following immune activation with Poly I:C, elicits both innate and adaptive immune response in the gut. The increase in pro-inflammatory cytokines and activated T cell markers indicates that mTG can activate the intestinal immune system, suggesting its potential as an immunogenic antigen capable of contributing to gut inflammation under certain conditions.

PT31

The gut microbiome and diet composition of Papua New Guinea Rodents among different altitudes from two mountain ranges

Presenter name: **Halil Mert Solak**

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Papua New Guinea rodents offer a rare example of mammalian adaptive radiation and a unique model for exploring how microbiota facilitate rapid niche expansion. We sampled over 800 individuals representing 45 taxa from two tribes (Rattini and Hydromyini) across five altitudes (200–3,700 m a.s.l.) in two PNG mountain ranges. Using 16S and 18S rRNA metabarcoding, we characterized gut bacterial communities and diet composition.

Gut microbiota diverged sharply between Rattini and Hydromyini, reflecting their independent colonization waves. Notably, invasive *Rattus rattus* and *R. exulans* harbored microbiotas more similar to Hydromyini than to native Rattini species. Invasive *Rattus* species also consume a more animal-based diet compared to their native counterparts. Within each tribe, microbial community composition was highly species-specific and was partly predicted by habitat type, host ecology, and elevation. Parallel diet shifts suggest linked ecological and microbial adaptations.

These findings establish a baseline for understanding how host taxonomy, environment, and diet jointly shape microbiome structure during adaptive radiation. They highlight the microbiome's potential role in driving rapid ecological diversification among free-living rodents.

PT32

Interspecific Divergence and the Effect of Neutral Processes on Microbiota Variation in Inbred Strains Derived from Eight Mouse Species

Presenter name: **Leighton J Thomas**

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To investigate microbiome differences attributed to host species, eight different mouse species, derived from a range of different inbred stains, were raised under identical laboratory conditions across many generations. Both species and strain effects strongly influenced alpha diversity, the effect size was larger in the oral microbiome with species accounting for 66% of deviance and 28.7% in caecal communities. Individuals derived from specific pathogen free (SPF) laboratories had significantly higher alpha diversity. A similar pattern was found in the analysis of beta diversity, with host factors exerting stronger effects in the oral microbiome than the caecum. The SPF derived strains had significantly different beta diversity. We applied neutral models to quantify how each species microbiome was shaped by neutral processes, overall the oral microbiome showed a bigger departure from neutrality than the ceacum microbiome, with the SPF strains showing the best fit to a neutral model. Across all species each individual ASV was found to be mostly consistent under the same selection pattern (positive selection, negative selection or neutral) with only a small number of ASVs being contradictory between species (>1%). A random forest model achieved 85% accuracy (95% CI: 70.16–94.29%; $\kappa = 0.83$) in classifying rodent species by microbiome profiles, and the top 50 discriminatory ASVs revealed distinct host-specific abundance patterns. These results demonstrate that host species maintain distinct microbiome profiles despite many generations of standardized rearing, and that SPF-derived mice still maintain a unique microbial community even after transfer to conventional facilities.

PT33

Making SPF mice great again: Symbiotic Microbiota Restoration in SPF Mice through Dirty Facility Exposure

Presenter name: **Jakub Kreisinger**

Co-authors: Dagmar Čížková (1,2), Barbora Bendová (1,2), Barbora Vošlajerová Bímová (3), Ľudovít Ďureje (2), Jaroslav Piálek(2), Lucie Schmiedová (1,2), Ondřej Štěpánek (4), Jakub Kreisinger (1)

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The specific-pathogen-free (SPF) house mouse is the standard model in biomedical research, but its deliberately simplified microbiota diverges sharply from that of wild mice, risking phenotype distortions. To test whether conventional housing can “re-wild” SPF microbiota, we translocated five SPF strains from three SPF facilities into two conventional facilities and sampled them after at least two generations, alongside wild-caught mice. We profiled caecal, ileal, and oral bacterial communities and assessed immune cell populations in the mesenteric lymph node.

Within two generations in conventional conditions, SPF mice’s microbiota nearly fully converged with wild-mouse communities, regardless of host strain. In contrast, mouse genetic background had only minor effects on microbial composition but remained the primary driver of immune-profile differences. Moreover, immune phenotypes differed only moderately between SPF and conventionally housed mice.

These results demonstrate that simple relocation to conventional facilities reliably restores a natural-like microbiota in SPF mice—without embryo transfer or fecal transplantation—while preserving genetic influences on immunity. Conventional housing thus offers an accessible “re-wilding” strategy to enhance the ecological validity of mouse models for host–microbiota interaction studies.

PT34

Advancing Gut Microbiome Metabolomics: A Derivatization-Based Approach

Presenter name: **Anna Michl**[a]

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Microbiota-associated metabolites play a crucial role in host physiology and disease by mediating host-microbiota communication, providing essential nutrients, and regulating metabolism and immune functions. Understanding these interactions requires comprehensive chemical analysis of the metabolites. However, their low abundance, diverse physicochemical properties, and complex matrices present significant challenges, leaving us with no universal method to analyze them all. Chemical derivatization is a powerful approach, particularly for polar metabolites. Modifying the analyte structure decreases their polarity and makes them compatible with conventional reverse-phase liquid chromatography.
3-nitrophenylhydrazine (3NPH) is a widely used derivatization agent, which was previously employed in studies targeting well-known microbial metabolites such as Short Chain Fatty Acids (SCFA). However, the potential of its applicability can be extended and go beyond to larger datasets, covering the majority of polar primary metabolites as well as known products of microbial metabolism.
Here, we present a large-scale analysis of nearly 600 gut microbiome-related chemical standards, derivatized using 3NPH. Standards were systematically classified based on structural similarities, biological relevance, and metabolic pathway associations using a Python-based automated categorization pipeline and public metabolomic databases. For streamlined identification, the recently introduced in-silico Derivatization Tool in MetaboScape by Bruker was employed for an automated targeted search.
To validate the applicability of derivatization strategy, we utilized the library of derivatized standards for the targeted analysis of microbiota-associated metabolites in stool samples from mice with different statuses of microbial colonization, including germ-free models. Our results demonstrate the utility of this approach in providing valuable insights into host-microbiota metabolic interactions.

PT35

Butyrate alters hepatic drug metabolism and expression of short-chain fatty acid transporters and receptors in the colon of mice with DSS-induced colitis.

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Introduction: Butyrate, a microbial metabolite produced by gut bacteria, exerts beneficial effects in colonic inflammation. Nevertheless, the precise molecular mechanisms through which butyrate acts remain incompletely understood.
Materials and Methods: Two-month-old BALB/c mice raised in specific-pathogen-free (SPF) conditions were assigned to experimental groups receiving sodium butyrate or serving as controls. Two different time regimens of butyrate administration were employed. Clinical signs such as body weight change, rectal bleeding, and prolapse were evaluated. Mucin production and colonic localization of the tight junction protein ZO-1 were analyzed. Plasma concentrations of IFN-γ, IL-6, and IL-10 were quantified. Furthermore, total RNA was isolated to assess hepatic expression of selected cytochrome P450 enzymes and colonic expression of short-chain fatty acid (SCFA) transporters and receptors.
Results: Pre-treatment with butyrate ameliorated intestinal inflammation and restored epithelial barrier integrity by re-establishing ZO-1 distribution in a DSS-induced colitis model. Butyrate also modulated hepatic expression of CYP3A subfamily members. In addition, it led to downregulation of SCFA transporters Slc16a1, Slc5a8, and Hcar2 in the colon of SPF mice. Expression of SCFA receptors was only partially affected by DSS exposure.
Discussion: These findings suggest that butyrate may influence therapeutic efficacy in inflammatory bowel disease by modulating both colonic and hepatic gene expression, including cytochromes P450 and SCFA-related pathways.

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PT36

Bifidobacterium longum ssp. *longum* CCM 7952-derived peptidoglycan alleviates allergic inflammation through TLR-dependent signaling in a mouse model of airway allergy

Presenter name: **Dagmar Srutkova[1]**

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Previously, we have shown that intranasal administration of heat-killed *Bifidobacterium longum* ssp. *longum* CCM 7952 (Bl7952), similar to live bacteria, retains the ability to suppress airway inflammation and alleviate allergy in OVA-sensitized and challenged mice (Pyclik et al., 2021). This finding prompted us to isolate specific Bl7952 cell wall molecules to identify those responsible for the anti-allergic effect.
Female BALB/c, C57Bl6, NOD2 KO and MyD88 KO mice were sensitized three times intraperitoneally with ovalbumin emulsified with the adjuvant alum. Four hours before each sensitization and before each of the four intranasal ovalbumin challenges, isolated Bl7952 antigens (slime exopolysaccharides, cell wall polysaccharides, surface proteins or peptidoglycan (PG)) were applied intranasally.
We show that of the molecules tested, only PG was able to reduce the total cell count and eosinophil count in BAL and lower the lung histopathology score compared to the PBS/OVA group. In addition, PG administration reduced the levels of OVA-specific IgE antibodies in both serum and bronchoalveolar lavage, serum levels of total IgE antibodies and levels of Th2-related cytokines in splenocyte cultures. *In vitro*, PG was able to signal via TLR2 and NOD2 receptors in the HEK293 cell reporter system. To determine whether either of these receptors is necessary for the observed allergy-reducing effect, we used NOD2 and MyD88 whole-body KO mice. The antiallergic effect of PG was maintained in NOD2 KO mice, but not in MyD88 knockout mice. This indicates that the PG effect is mainly mediated by the MyD88 adaptor molecule and thus TLR2 receptor.
These results suggest that Bl7952 PG has anti-allergic properties and may be a promising postbiotic candidate for further investigation.

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For PT37 abstract see LT13

PT38

Uncovering a novel acetosyringone catabolic enzyme with co-metabolic activity towards chlorinated pollutants

Presenter name: **Michal Strejček**[1]

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Acetosyringone, a prominent phenolic metabolite derived from S-lignin, plays a pivotal ecological role in shaping microbial communities through its antimicrobial and allelopathic properties. Despite its environmental relevance and structural resemblance to aromatic xenobiotics, little is known about its bacterial degradation pathways. In this study, we enriched a bacterial consortium on acetosyringone as the sole carbon source and identified a dominant strain, *Pseudomonas rhizophila* AS1, capable of initiating acetosyringone catabolism. Metagenomic reconstruction and functional screening uncovered a novel FAD-dependent monooxygenase, AsdA, that directly hydroxylates the aromatic ring of acetosyringone, representing a previously undescribed initiation mechanism distinct from side-chain modifications known in other acetosyringone degrading bacteria.

Remarkably, AsdA also catalyzes the initial transformation of 2,4,6-trichlorophenol, a persistent environmental pollutant, establishing its dual functionality in both natural and anthropogenic compound turnover. Expression of *asdA* in *Escherichia coli* demonstrated acetosyringone and trichlorophenol transformation capacity, and non-targeted LC-MS/MS analysis provided mechanistic insights into its catalytic versatility. The use of constructed biosensor strains confirmed that acetosyringone serves as a strong inducer of the *asd* gene cluster, providing a functional link between lignin-derived compounds and chlorinated pollutant degradation.

Together, these findings establish acetosyringone not only as a valuable probe substrate for discovering catalytically promiscuous enzymes but also highlight AsdA as a potential biocatalyst for lignin valorization and bioremediation strategies.

PT39

Genomic characterization of groundwater bacterial phylum UBA9089

Presenter name: **Roman Skala**

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Groundwater ecosystems remain largely unexplored, yet they harbour diverse and previously undescribed prokaryotic lineages. Many of these inhabitants of the deep biosphere are challenging or impossible to cultivate in a laboratory setting, necessitating the use of cultivation-independent approaches for their study, such as metagenomics. This approach enables the sequencing of collective genetic material directly from environmental samples, followed by the assembly of individual genomes from these metagenomes and their subsequent in silico analysis. Metagenomic analysis of the Crystal Geyser in Utah, USA, led to the discovery of a novel bacterial phylum named UBA9089. Subsequent research has revealed that members of UBA9089 inhabit diverse oligotrophic, anoxic groundwater environments worldwide, including CO_2-saturated geysers, uranium mine waters and thermal springs, some of which are used for spas. Metabolic predictions based on a limited number of metagenome-assembled genomes (MAGs) suggest that UBA9089 is capable of sulphate reduction and utilises the Wood–Ljungdahl pathway for carbon fixation or organic compound degradation.
This work expands our understanding of UBA9089 by profiling its environmental distribution across publicly available metagenomes and assembling new genomes to increase taxonomic representation. A broader genome set enables more precise metabolic predictions, indicating a widespread Wood–Ljungdahl pathway and hydrogen oxidation coupled to sulfate reduction, as well as the potential for extracellular iron reduction in certain genomes. Finally, we explore the phylogenetic relationships within the UBA9089 phylum and its position among neighbouring taxa.

PT40

Harnessing Microbial Metabolism: A Conjugative Plasmid-Based Strategy to Enhance Degradation of Organic Contaminants in Soils

Presenter name: **Jáchym Šuman**

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Due to the immense metabolic diversity and versatility, microorganisms represent a powerful tool for removing organic pollution in the environment, including (poly)aromatic hydrocarbons and their derivatives. The use of promiscuous conjugative machinery to spread corresponding genetic determinants within indigenous microbial communities may boost their degradation capacity. This work aims to demonstrate the concept using an advanced synthetic biology approach that combines the toluene dioxygenase (*tod*) gene cluster from *Pseudomonas putida* F1 with the RP4-derived conjugative plasmid pMATING2α. The *tod* gene cluster was inserted into pMATING2α, yielding pMATING-tod, which enables green fluorescent protein-based detection and selection of transconjugants. Engineered *Pseudomonas putida* EM42-derivative was used to deliver pMATING-tod into the soil microbial community. In a pilot experiment with common grassland soil, pMATING-tod-bearing transconjugants were plate-recovered, and their naphthalene degradation capacity was demonstrated. This study serves as proof of concept for enhancing the bioremediation efficiency of indigenous microbial communities. We propose the application of such a system across diverse environmental matrices, including soils, groundwaters, wastewaters, and sludges.

PT41

Stable isotope probing to disentangle carbon for nitrogen trading in mycorrhizal hyphosphere

Presenter name: **Petra Bukovská**

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There is experimental evidence for the key role of hyphosphere microbiomes in releasing mineral nutrients such as nitrogen (N) from organic moieities in soil to be accessible for the arbuscular mycorrhizal (AM) fungi and their host plants, in consequence. Yet whether and how the N (or access to it) is exchanged by the hyphosphere microorganisms for reduced carbon (C) supplied by the AM fungus remains unclear. To disentangle whether and which microbes involved in organic N (chitin) degradation are reciprocally rewarded by fungal C, we undertook several stable isotope probing experiments, labeling both the AM fungus or the chitin with ^{13}C, or chitin only with ^{15}N. We followed the incorporation of the heavy isotopes into the microbial (prokaryotic) RNA in microbially complex microcosms. Our results so far indicate that microbes could utilize the chitin as sources of both N and C, not necessarily relying on the AM fungus to supply the energy/carbon. On the other hand, evidence was obtained for specific microbial taxa (e.g., *Aquicella*, *Reyranella*, *Chitinophaga*, or *Devosia*) to be preferentially occurring in the AM fungal hyphosphere. Yet, whether these taxa specifically occupy the hyphoplane or are more loosely associated with the fungus still needs to be addressed. Importantly, only a handful of prokaryotic taxa were enriched with both ^{15}N from the chitin and ^{13}C from the living AM hyphae, and not by the ^{13}C from chitin, which indicates that relying on fungal carbon while mineralizing organic N is possibly a rare ecological strategy.

PT42

Disentangling mycorrhiza-nitrification interactions in soil by using synthetic nitrification inhibitors

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Arbuscular mycorrhizal fungi (AMF) and ammonia oxidizing (AO) microorganism interactions could significantly shape soil nitrogen (N) cycling and plant N acquisition, but experimental evidence for this is still scarce. Here we used a novel experimental design, supplying living field soil into a root-free compartment in pots, where mycorrhizal or nonmycorrhizal plants were grown, thus exposing the soil indigenous microbes to influence of the proliferating AMF hyphal networks – or not. The soil patches in the root-free zone were amended with $^{15}NH_4Cl$ to trace the fate of the N and treated with nitrapyrin or ethoxyquin to suppress AO bacteria or archaea, respectively, as compared to no nitrification inhibitor amendment. The abundance of AO archaea was suppressed by AMF and by both inhibitors. In contrast, AMF had no effect on the abundance of AO bacteria. Moreover, whereas nitrapyrin strongly suppressed their abundance, ethoxyquin significantly stimulated the abundance of AO bacteria, possibly through competitive release. This probably was the reason why nitrapyrin nearly blocked ^{15}N transfer from soil to nonmycorrhizal plants. Yet, amendment with ethoxyquin had almost no effect on this passive ^{15}N transfer pathway. The AMF facilitated much larger ^{15}N transfer from soil to plants as compared to the passive pathway, and this was slightly suppressed by ethoxyquin compared to nitrapyrin. These results indicate major role of AO bacteria in soil N cycling upon absence of AMF. The role of AO archaea is less pronounced, as is the direct and indirect effects of ethoxyquin on the mycorrhiza-mediated N uptake by the plants.

PT43

Short-term biocide stress alters soil microbial communities and affects plant growth, carbon stabilization, and enzymatic activity in soil

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Soil microbial diversity sustains key ecosystem functions, and the simplification of soil microbial communities may compromise ecosystem processes and stability. To investigate this, we conducted a two-stage experiment in which eight biocides (four antibiotics, four fungicides) were used to suppress specific microbial guilds. The first stage aimed at imposing short-term biocide stress on a complex soil microbial community and measuring their population size and metabolic activity (respiration of ^{13}C-glucose) upon incubation in closed vessels. Antibiotic treatments induced a stronger disturbance, reflected in higher $^{13}CO_2$ release than fungicides, and qPCR showed that biocides affected mostly bacterial and protistan, but not fungal, communities. Based on the qPCR results, ceftazidime was less effective in suppressing the microbial abundance. In the second stage, we introduced these pre-treated communities into a plant bioassay, assessing growth of mycorrhized and non-mycorrhized chicory in different pre-incubated soils and measuring soil processes. Mycorrhiza suppressed plant biomass production, contrary, biocides generally increased plant biomass production. Furthermore, the presence of mycorrhiza improved the stabilization of carbon in the soil substrate. Soils treated with the fungicides myclobutanil and kasugamycin had higher rates of stabilized ^{13}C compared to soils treated with antibiotics. Both chitinase and acid phosphatase activities were higher in mycorrhizal treatments. Besides, chitinase activity was positively affected by pre-treatment of microbes with the fungicide cycloheximide. These findings indicate that the biocide pre-treatments significantly influenced the structure of soil microbial communities, resulting in changes in plant biomass and soil ecosystemic processes.

For PT44 abstract see LT14

PT45

GlobalFungi - the global atlas of fungal biodiversity

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The introduction of high-throughput sequencing has significantly advanced our ability to monitor microbial distribution in a wide range of ecosystems and geographical areas. The creation of the FAIRified repository of published observations – the GlobalFungi database aims to improve access to and interpretation of global data on fungal occurrences in the environment, facilitating the study of fungal biodiversity and biogeography. The GlobalFungi database (https://globalfungi.com) compiles over 4.5 billion observations of fungal metabarcoding markers – the internal transcribed spacer regions ITS1 and ITS2 - from 846 studies, covering 84,972 samples of fungi in soils, litter, air, aquatic habitats, plant material and decomposing deadwood. The database web interface enables data searching and visualization as well as data and metadata downloads and promotes the community of soil biodiversity researchers to submit their data to this common repository available for the scientific community. The GlobalFungi database represents a significant advancement in mapping the global diversity and distribution of fungi. So far, it was used to generate global biodiversity maps, to estimate the global species diversity of fungi or to identify environmental constraints on fungal distributions offering a comprehensive perspective on fungal ecology, biogeography, and the environmental factors that influence fungal distribution, encompassing all ecologically relevant fungal groups.

PT46

GlobalAMFungi – the global atlas of arbuscular-mycorrhizal fungal biodiversity

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The advent of high-throughput sequencing has revolutionised our ability to trace the biogeography of arbuscular-mycorrhizal (AM) fungi—key plant symbionts that underpin nutrient acquisition, stress tolerance and global carbon cycling. Yet until recently, AM-fungal occurrence data were scattered across hundreds of case studies, limiting synthetic analyses and large-scale ecological inference.

To overcome this bottleneck, we established GlobalAMFungi (https://globalamfungi.com), a FAIR-compliant, open-access repository that harmonises and georeferences almost 90 million Glomeromycotinian amplicon observations drawn from 8 464 environmental samples reported in 100 independent metabarcoding studies spanning 108 countries. Sequencing reads originate from three barcoding loci—SSU rRNA, ITS2 and LSU rRNA—thereby capturing the marker heterogeneity typical of AM-fungal surveys.

An intuitive web interface lets users query taxa, visualise distribution maps and download variant sequences and rich environmental metadata for soils, roots, sediments and other substrates. The platform is designed for continuous community curation and automatic versioning, ensuring transparent provenance and reproducibility.

GlobalAMFungi has already been leveraged to produce global richness estimates, to reveal climate- and pH-driven turnover in AM-fungal assemblages and to guide conservation priorities for below-ground biodiversity. By consolidating diverse data sources into one comprehensive repository, the database delivers an unprecedented foundation for tackling fundamental and applied questions in AM-fungal ecology, evolution and ecosystem management at truly planetary scales.

PT47

Interaction of Silymarin Flavonolignans with Human Gut Microbiota

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Silymarin, an extract from fruits of the milk thistle *Silybum marianum* (L.) Gaertn., is made up of the flavonolignans silybin A and B, isosilybin A and B, silychristin A and B, silydianin and the flavanol taxifolin. Minor components include isosilychristin, 2,3-dehydrosilybin, 2,3-dehydrosilychristin and 2,3-dehydrosilydianin. The bioavailability of silymarin components is 20–50% due to low. The aim of the study was to evaluate the complex interactions of silymarin and its components with gut microbiota both *ex vivo* and *in vivo*. Biotransformation of silymarin components was studied ex vivo, using batch incubations inoculated by fecal slurry and HPLC/MS. Using advanced techniques NGS, NMR and LC-MS, we analyzed the dual impact of the microbiome on silymarin metabolism and the effect of silymarin on the microbiome's structure and function. Finally, in 33 healthy adult male volunteers, who received 200 mg of silymarin orally twice daily for three months, formation of metabolites in urine and feces was evaluated by HPLC/MS and bacterial composition of feces was investigated by NGS. At 200 mg/L the flavonolignans were resistant to the metabolic action of microbiota. At 10 mg/L, biotransformation of flavonolignans was much slower than that of taxifolin. Silybin, isosilybin, and 2,3-dehydrosilybin underwent mostly demethylation, silychristin was predominantly reduced. Silydianin, 2,3-dehydrosilychristin and 2,3-dehydrosilydianin were reduced and decarbonylation and cysteine conjugation proceeded. No low-molecular-weight phenolic metabolites were detected. Silymarin significantly altered the metabolism of the gut microbiota, decreasing short-chain fatty acid production and glucose utilization. Healthy elders (70–80 years) showed a significant increase in a specific catabolite associated with *Oscillibacter*. Conversely, healthy young donors (12–45 years) exhibited faster breakdown of silymarin components, particularly isosilybin B, which negatively correlated with higher abundance of *Faecalibacterium* and *Erysipelotrochaceae UCG-003*. In volunteers, a correlation was found between the number of metabolites and the composition of the intestinal microbiota.

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PT49

Re-Wilding Laboratory Mice: Colonizing Germ-Free C57BL/6 to Establish Lines with Wild-Derived Gut Microbiota.

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Laboratory mice are usually maintained under specific pathogen-free (SPF) conditions, which markedly alter their gut microbiota (GM) compared to wild counterparts. As a result, SPF mice often show atypical host‑microbiota interactions, including increased susceptibility to some experimentally induced diseases. These limitations raise concerns about the relevance of SPF-based models for studying GM‑host interactions and microbiota-influenced traits. To address this, "re-wilding" approaches have been proposed–colonizing laboratory mice with natural microbiota from wild donors to better approximate ecological and evolutionary host‑microbiota relationships.

We colonized female germ-free C57BL/6 mice with wild-type GM from four wild-derived house mouse strains maintained in a conventional facility without SPF interventions. Our goals were to compare two colonization methods–oral gavage and co-housing–with respect to mortality and colonization dynamics, and to establish C57BL/6 lines carrying wild-type GM.

Ten of 14 germ-free females survived colonization, with no difference in mortality between the two methods. Each was sampled repeatedly over 50 days, and GM temporal dynamics are being analyzed. To date, four C57BL/6 lineages harboring wild-type GM have been established. These lines advance the re-wilding of laboratory mice and provide new models for studying host‑microbiota interactions under more natural conditions.