1st SLOVENIAN MICROBIOME NETWORK SYMPOSIUM

November 24, 2022
Bled, Slovenia

1st Slovenian Microbiome Network Symposium
2022, Bled, Slovenia

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Polonca Štefanič, University of Ljubljana, Slovenia
Primož Treven, University of Ljubljana, Slovenia

Content

Welcome note

Dear colleagues,

Welcome to the 1st Slovenian Microbiome Network Symposium.

The field of microbiome research has developed rapidly over the last decades. New and exciting findings about microbiomes, their systemic role, interactions, and their impact on the host or the environment are generated on a daily basis. However, the application of microbiomes and their functions as a basis for a sustainable, circular bioeconomy still seems to be a long way off. Nevertheless, significant progress can be made if the knowledge gained in different fields is combined to develop reliable predictive models and mechanisms that can be easily applied to microbiomes from different environments to perform desired functions.

We, therefore, organized the Slovenian Microbiome Network Symposium to strengthen collaboration and promote knowledge transfer between fields at the national and international levels. This first symposium will provide a broader overview of microbiome research in different fields and will serve as a starting point for future annual symposia, which will be thematically focused.

We have invited leading scientists to present pressing issues in the broad fields of microbiology, microbial ecology, genomics, and complementary disciplines. The program is suitable for participation of early- and mid-career scientists who wish to present and extend their research. We have organized the symposium as a single-session event, which will certainly allow the promotion and dissemination of the research presented to a truly diverse audience. Opportunities for discussion and networking are available throughout the symposium; at the poster session, during coffee breaks, and at the end at the dinner reception.

We hope that the symposium will offer you many opportunities to interact and share your work with scientists in the field.

On behalf of the organizing committee, I wish you a fruitful and interesting meeting.

Nejc Stopnišek

PROGRAM

9:00 - 9:15 Opening talk note

9:15 - 11:15 Section 1: ENVIRONMENTAL MICROBIOME (Nataša Šibanc and Blaž Stres)

9:15-9:55 Tina Šantl-Temkiv: Atmospheric microorganisms: assembly, activity and climate impacts

9:55-10:20 Tinkara Tinta: Marine microbiomes in the sea of change

10:20-10:45 Marjetka Suhadolc: Soil microbiomes in light of sustainable agriculture and climate change mitigation – what can be learned from long-term field experiments?

10:45-11:00 Tine Grebenc: Do fungal and bacterial communities in ascocarps shape the arome of true truffles

11:00-11:15 Irena Maček: Arbuscular Mycorrhizal Fungi – Ancient Organisms That Live at the Interface Between Plant Roots and Soils

11:15 - 12:00 POSTER SESSION with COFFEE BREAK

12:00 - 13:00 LUNCH BREAK

13:00 - 15:15 Section 2: HOST MICROBIOME (Aleksander Mahnič and Polonca Štefanič)

13:00-13:40 David Berry: Starting off on the right foot: Microbiome and gut-brain axis development in extremely premature infants

13:40-14:05 Maja Rupnik: Gut microbiota, Clostridioides difficile and beyond

14:05-14:30 Denis Kutnjak: Characterisation of plant viromes: from crops and wild plants to environmental waters

14:30-14:45 Gorazd Avguštin: Differences in gut microbiome of lean and fat mice and changes related to nutritional shifts

14:45-15:00 Martina Reberšek: Gut microbiome and systemic therapy in oncology

15:00-15:15 Sandra Hudina: Changes in the microbiome along the invasion range of a successful freshwater invader

15:15 - 15:45 COFFEE BREAK

15:45 - 17:45 Section 3: ENGINEERED MICROBIOMES (Primož Treven and Tomaž Accetto)

15:45-16:20 Michael Schloter: Healthy Soils – Healthy Humans? The Role of Microbiomes in the One Health Concept

16:20-16:45 Monika Novak Babič: Household microbiomes - a 10 years summary

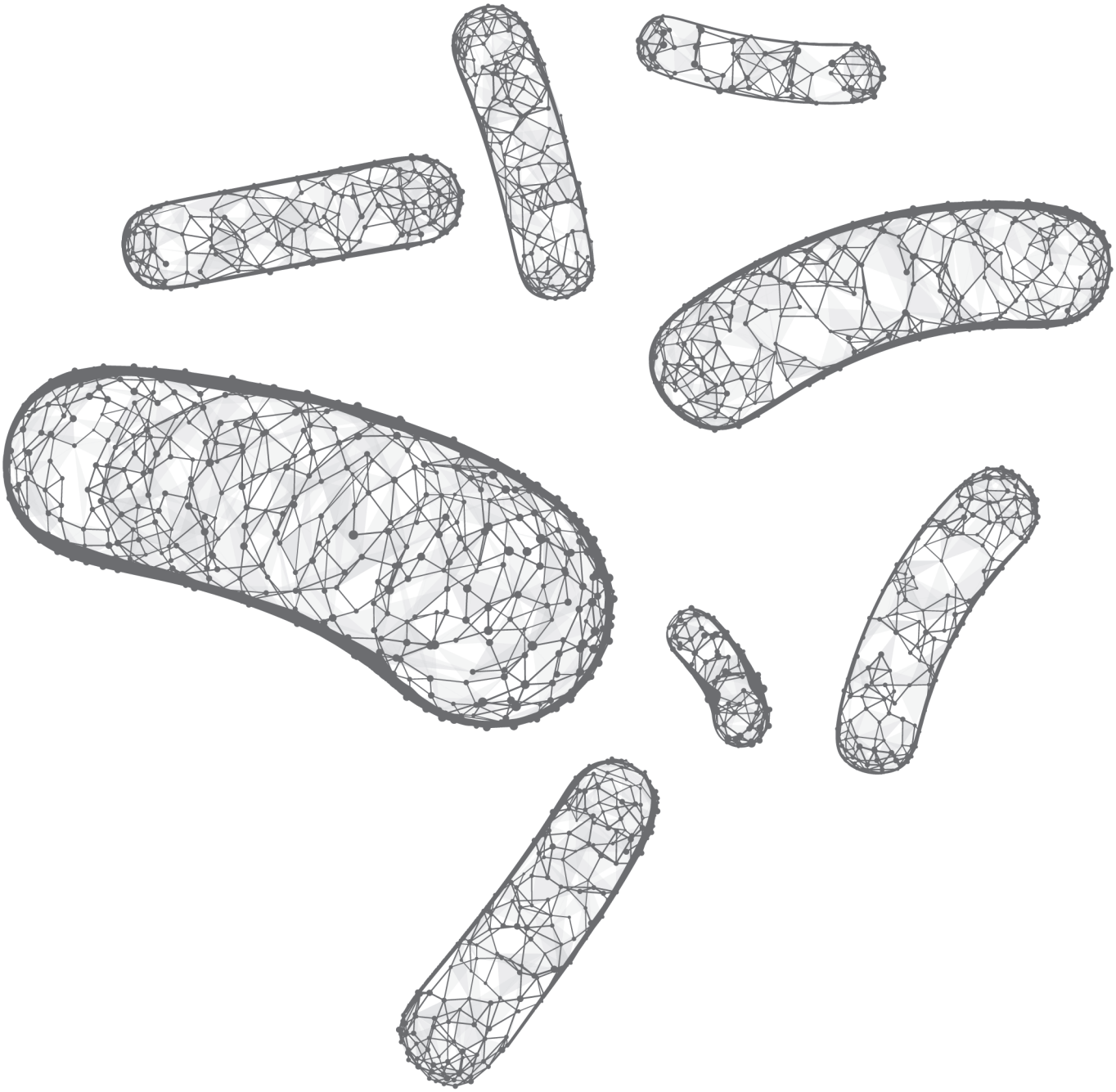
16:45-17:00 Polona Zalar: Microbiomes of Slovenian cultural heritage objects

17:00-17:15 Jure Mravlje: Cold plasma treatment alters fungal community structure of buckwheat grains

17:15-17:35 Brane Leskošek: ELIXIR-SI research infrastructure – an overview of tools and services

17:35-17:45 CLOSING REMARKS

18:00 DINNER RECEPTION



PRESENTATION OF KEYNOTE SPEAKERS

Tina Šantl-Temkiv

Department of Biology - Microbiology
Department of Physics and Astronomy
iCLIMATE Aarhus University Interdisciplinary Centre for Climate Change
Arctic Research Centre
Aarhus University
Aarhus, Denmark

TALK TITLE:
Atmospheric microorganisms: assembly, activity and climate impacts

I have been working within the field of atmospheric microbiology for the past 14 years. I did my PhD on this topic at the National Environmental Research Institute, Roskilde, Denmark. Afterwards, I continued with a 4-year postdoc position as part of the Stellar Astrophysics Center at Aarhus University (AU), where I linked the study of atmospheric microorganisms with astrobiology and the search for life on exoplanets. After doing a postdoc in the aerosol laboratory at Lund University, I have been heading the group for atmospheric microbiology as an assistant professor at the Department of Biology (AU) since 2016. I focus on the activity of atmospheric microorganisms, their source environments, and their impact on the formation, properties and lifetime of clouds.

David Berry

Division of Microbial Ecology Department of Microbiology and Ecosystem Science
University of Vienna
Joint Microbiome Facility
Vienna, Austria

TALK TITLE:
Starting off on the right foot: Microbiome and gut-brain axis development in extremely premature infants

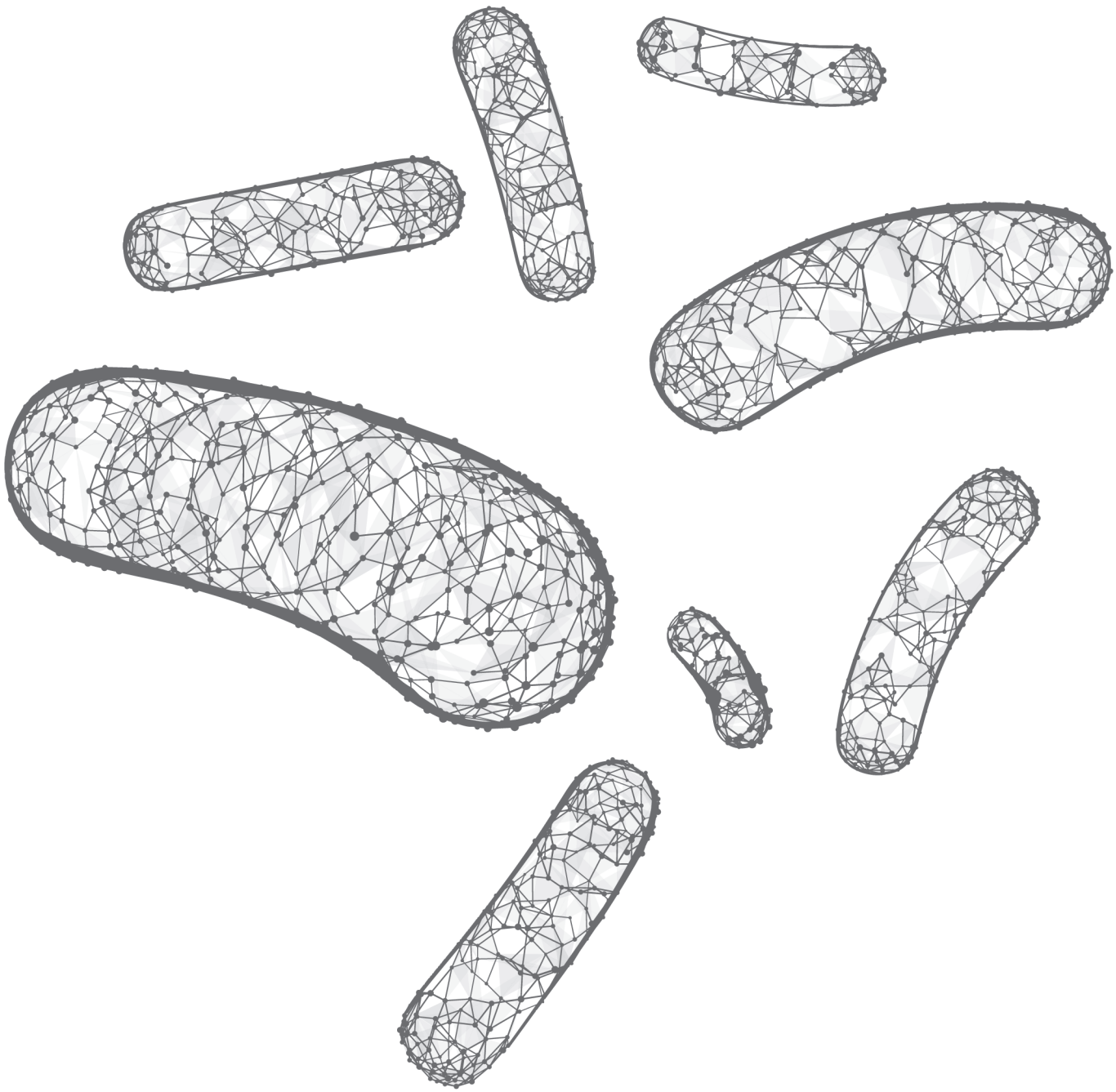
David Berry is a professor at the University of Vienna, where he leads a research group focused on the ecology and evolution of the gut microbiota. He is also Operational Director of the Joint Microbiome Facility, which provides sequencing-based microbiome analyses for environmental, pre-clinical, and clinical studies. He holds a Ph.D. and M.S.E. in Environmental Engineering from University of Michigan, and B.S. in Bioresource Engineering from Rutgers University. His group's research focuses on the function of the gut microbiota in health and disease, with particular interest in inflammation, cancer, and neurodevelopment. In addition to studying the ecology and evolution of key species in the gut microbiota, a major focus is to develop and apply novel molecular and isotope-labeling methods for studying uncultivated microorganisms in their natural environment.

Research Unit for Comparative Microbiome Analysis, Helmholtz Center Munich
School of Life Sciences, Technical University Munich
Munich, Germany

Michael Schloter

TALK TITLE:
Healthy Soils – Healthy Humans? The Role of Microbiomes in the One Health Concept

Professor Schloter is director of the Research Unit for Environmental Genomics at the Helmholtz Center Munich. He studied biology in Munich and received his PhD in 1994 from the University of Bayreuth. The main focus of his work is the examination of the genetic diversity of microflora in different environmental compartments. Furthermore, Professor Schloter is involved in several human microbiome initiatives. His main goal is to understand the interaction between microbes and its meaning for ecosystem functionality and performance. To achieve his goals Professor Schloter uses modern high throughput sequencing technologies. Michael Schloter's research group has published more than 150 papers in the last 5 years. Since 2001 professor Schloter has been teaching at TUM in the Department of Life Sciences. He was appointed honorary professor of microbiology in 2010.



ABSTRACTS OF INVITED AND SELECTED ORAL PRESENTATIONS

INV1

Marine microbiomes in the sea of change

Tinkara Tinta

Marine Biology Station Piran National Institute of Biology Piran, Slovenia

Oceans and seas constitute the largest part of Earth's biosphere, and their smallest inhabitants – microbes - are invisible to the naked eye, but account for most of the ocean's biomass and are the most productive and diverse members of marine food webs. The key factor contributing to the cosmopolitan distribution of microbes in oceanic habitats is the plasticity of their genomes (and thus their adaptability) and the different types of metabolic processes, some of which unique to microbes. In seawater, the pool of organic matter (OM) is composed of a plethora of chemical compounds of different origins, characterized by different size, complexity, availability, and reactivity. Through a chain of biochemical reactions, all particulate OM is eventually transformed into dissolved OM, which is almost exclusively accessible to microbes that operate various metabolic networks, and in this way, drive biogeochemical cycles at the base of the oceanic food web. The abundance, metabolic activity, and composition of microbial communities are influenced by a range of physical, chemical, and biological parameters, as well as by various interactions among organisms. Marine microbes are subject to constant fluctuations in environmental parameters, especially in coastal areas, and the resulting changes in microbial community dynamics affect the structure and functioning of marine ecosystems. Natural and anthropogenic influences are paving the way for projected changes in future oceans and will likely inevitably impact marine life, microbes, and the biogeochemical cycles they drive. However, the complexity, underlying mechanisms, and consequences of these processes remain unclear. Recent methodological advances in analytical chemistry and "omics" technology are providing insights into the relationship between individual compounds within the complexity of the OM pool and the metabolic network operated by the microbial community under specific environmental conditions. Only when we have gained this mechanistic understanding will we be able to predict the response of the marine ecosystem to natural and anthropogenic perturbations.

I am a marine microbiologist studying the effects of natural and anthropogenic disturbances on microbial community dynamics in marine ecosystems. My research is interdisciplinary and draws on my background in biochemistry (BSc) and my area of expertise – marine microbial ecology (PhD). During my PhD, I was a FEMS fellow at Lund University, Sweden. For my postdoctoral research at Scripps Institution of Oceanography (UCSD, USA) I received a Fulbright Fellowship. My research at the University of Vienna, Austria, was funded by a Marie Skłodowska-Curie Individual Fellowship. Currently, I am working at MBP-NIB, which is located in a coastal area subject to constant fluctuations in environmental parameters and anthropogenic impacts, with increasing blooms of certain organisms and various types of pollution. Here, we focus our research on the interactions between the coastal microbiome and bloom-forming jellyfish and microbial indicators of faecal pollution, including potential pathogens, and impacts on marine biogeochemical cycles.

INV2

Soil microbiomes in light of sustainable agriculture and climate change mitigation – what can be learned from long-term field experiments?

Marjetka Suhadolc

Biotechnical Faculty, University of Ljubljana

Soil microbial communities are major drivers of the nutrient cycling that sustain plant growth and productivity. In addition, soil microbes influence climate through carbon storage and greenhouse gases (GHG) production and consumption. Yet, the effects of soil management practices on the soil microbiome are still poorly understood. Here, results from several field experiments that examined the long-term consequences of changes in soil management practices (tillage intensity, fertilization strategy, crop rotation, permanent vegetation cover) on the soil microbial community composition and functioning will be presented. An integrated understanding is urgently needed to apply best practices for sustainable soil management to support crop production, store and supply clean water, maintain biodiversity, sequester carbon, reduce soil GHG emissions, and increase resilience in a changing climate.

Marjetka Suhadolc, Ph.D., is an Associate Professor. of Soil Science at the Biotechnical Faculty of the University of Ljubljana and has (co)designed three agroecology and environmental protection courses that did not exist before the Bologna reform. Marjetka has more than 20 years of research experience in soil ecology. Her main research interest is the relationship between environmental factors (pollution, climate) as well as soil management practices (tillage, fertilization, soil remediation) on the composition and functioning of soil microbial communities (C and N-cycles). She has participated as national coordinator in several EU projects: FOOTPRINT, EcoFINDERS, CoreOrganic Plus – FertilCrop, and several internal projects (CarboSeq, Minotaur, EnergyLink) of the EJP Soil Program (https://ejpsoil.eu/). She is actively involved in the COST action EUdaphobase - European Soil-Biology Data Warehouse for Soil Protection.

INV3

Gut microbiota, Clostridioides difficile and beyond

Maja Rupnik

National laboratory for health, environment and food (NLZOH), Faculty of Medicine, University of Maribor, Maribor, Slovenia

Clostridioides difficile infection is typically associated with disbalanced gut microbiota and several mechanisms of colonization resistance are well described. We have used a simple in vitro co-culture/ spent medium system to investigate interactions between various types of C. difficile strains and various types of gut microbiota. In addition to known ways on microbiota effects on C. difficile, our results show that C. difficile can specifically modulate gut microbiota and effects are more elaborate on dysbiotic microbiota. Dysbiotic microbiota also influence C. difficile sporulation. Both inhibition of healthy microbiota restoration and increased sporulation enhance C. difficile spore transmissions. We have investigated the effect of polyphenol modulation of microbiota on colonization resistance against C. difficile. Most types of blueberry polyphenol modulated microbiota lost the colonization resistance and C. difficile growth was comparable to antibiotic modulated controls. Other polyphenols (pomegranate) also resulted in microbiota supportive of C. difficile growth but decreased the cytotoxicity. C. difficile is often isolated from patients with IBD, another condition associated with chronic disbalance in gut microbiota and we have also investigated microbiota in gastrointestinal patients with or without IBD. Recently, our studies expanded beyond C. difficile. We have described gut microbiota in healthy Slovenian cohort. Subsequently, this cohort was compared with gastrointestinal hospitalized patients. Results suggested that some of the previously described 'disease specific' microbial signatures are likely associated with more general types of dysbiosis found across various diagnoses. We are developing cultivation approaches to study specific bacterial groups (gluten degraders, Faecalibacterium sp.,) virome (description of new bacteriophages) and longitudinal stability of bacteria and viruses. We are also developing animal fecal microbiota database for various further applications.

Maja Rupnik is head of Department for microbiological research at National laboratory for health, environment and food (NLZOH) in Maribor, Slovenia. Also, since 2005 she has been a Professor for Microbiology at Faculty of Medicine, University of Maribor. She is a worldwide recognized expert on Clostridium difficile and one of the main organizers of traditional event International Clostridium difficile Symposium (www.icds.si). Infection with C. difficile is typically associated with disturbed gut microbiota and her group is developing diverse approaches to study C. difficile interactions with microbiota. The methodological knowledge is also transferred to analyses of complex populations in clinical relevant and other materials. She was the president of Slovenian microbiological society from 2016 to 2020. The honors and awards she obtained for her work include Alexander von Humboldt grant, ESCMID/bioMerieux Award for Advances in Clinical Microbiology and national award Zois Certificate of Recognition for exceptional scientific achievements in microbiology.

INV4

Characterisation of plant viromes: from crops and wild plants to environmental waters

Denis Kutnjak

Department of Biotechnology and Systems Biology, National Institute of Biology (NIB), Ljubljana, Slovenia

Plant viruses have traditionally been studied in the context of disease they cause in crops, using targeted detection methods. In a recent decade, high-throughput sequencing technologies have allowed untargeted studies of all the viruses associated with a specific sample type, i.e., virome studies. Many new viruses were discovered in crop and wild plants and virome studies have demonstrated the influence of land usage on the diversity of plant viruses in an ecosystem. We have used virome approaches to establish untargeted detection of plant viruses in crop and water samples and extend this knowledge to better understand the occurrence and diversity of plant viruses in an ecosystemic context. In a recent study, we have investigated the virome of tomato, wild plant species, irrigation and surface water samples from 14 tomato farms in Slovenia. In plants, we uncovered 37 known and close to 80 novel viruses, most of the latter found in wild plants' viromes. These finding demonstrated that diversity of plant viruses is still very much understudied, even in better-characterized systems, such as tomato agroecosystem. High diversity of plant viruses was also observed in investigated water samples. Some known and novel viruses were detected in different sample types, implying their persistence and/or putatively wide distribution in an ecosystem. Our future research efforts are directed in better understanding the diversity and possible flux of plant viromes in an ecosystem, e.g., from urban waste to associated water bodies, wild and crop vegetation.

Denis Kutnjak is employed at the National Institute of Biology (NIB), Department of Biotechnology and Systems Biology, Ljubljana, Slovenia, from 2012 on, currently as a Scientific Associate. From 2021 on, he is leading a research unit Microbiology within the Department. His research interests include studies on diversity, evolution and epidemiology of viruses. He uses modern and classical virological tools to study viromes of plants, animals and environmental samples. He is specialized in the use of high-throughput sequencing and bioinformatics in virology and is transferring those skills also into other fields, such as virus diagnostics in plants and wastewater, as well as into studies focusing on environmental DNA.

INV5

Household microbiomes - a 10 years summary

Monika Novak Babič

Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

People spend ~90% of their time indoors, where they are exposed to various microbes through drinking, showering, cooking and recreational activities. Over the past decade, research on the household microbiomes revealed microbial communities in drinking water, kitchen surfaces, dishwashers, and washing machines. Detection methods have included water filtration with 20- and 45- µm pore filters, surface swabbing with sterile cotton swabs, isolation of pure cultures of fungi and bacteria on selected culture media (MEA+Ch, DRBC, HA, and R2A), and permanent storage of strains in the Ex-culture collection (IC Mycosmo). Identification of individual strains based on DNA extraction, amplification and sequencing of the ITS region, actin, beta-tubulin and translation elongation factor for fungi, and 16S rDNA for bacteria. Next-generation sequencing using the 454-platform and Illumina was used to study the microbial communities of drinking water and dishwasher samples. Synergism-antagonism of culturable bacteria and fungi was tested after isolating individual strains from 1 cm2 surface area of dishwasher rubber seals. The strains with the best synergistic potential were used for biofilm formation on different materials and in survival tests against physico-chemical agents. Culturable and molecular methods revealed the water-transmitted origin of the microbial communities on kitchen surfaces and household machines. Another part of the microbiota colonizing machines originates from food, textiles, skin and mucosa. Studies also suggest that high temperatures, humidity, desiccation, detergents, organic residues, and building materials lead to the selection and overgrowth of opportunistic microbes with potential impacts on human health. For example, synergistic microbes from dishwashers successfully formed biofilms in vitro and were sturdier when exposed to washing at higher temperatures, oxidative stress and mechanical removal. The results of household microbiomes revealed overlooked niches for opportunistic microorganisms, paved the way for changes in drinking water regulations, and were used in collaborations to improve the construction of household machines.

Monika Novak Babič studied microbiology in pre-Bologna programme at Biotechnical Faculty, University of Ljubljana, Slovenia. Her thesis focussed on polyextremotolerant fungi from dishwashers and washing machines. She continued her study in the PhD programme Biomedicine (sub-programme Microbiology) at the same University, with a research focus on fungi from indoor wet niches, including opportunistic fungi from drinking water and groundwater. She is currently assistant researcher at the University of Ljubljana, Biotechnical Faculty, Biology Department and continues her post-doctoral research on fungal ecology and diversity in drinking water. Search targets include linking medically relevant fungi with their presence in water, and implementation of fungal parameters in monitoring of water. Other lines of work include experimenting, consulting and writing expertise for industry producing clean water and household products.

INV6

ELIXIR-SI research infrastructure – an overview of tools and services

Polonca Ferk, Brane Leskošek, Jan Gojznikar, Marko Vidak, Nadja Žlender

Centre ELIXIR-SI, Institute for Biostatistics and Medical Informatics, Faculty of Medicine, University of Ljubljana Ljubljana, Slovenia

ELIXIR-SI is a Slovenian national research node that supports wet-lab and bioinformatics research infrastructure (RI) in many different research areas e.g. metagenomics, microbiome, personalized human and veterinary medicine, systems biology, digitalisation and robotisation of agriculture, plant sorts and plant diseases. ELIXIR-SI is the part of ELIXIR european network for distributed infrastructure in life sciences (https://elixir-europe.org).
The ELIXIR-SI RI was upgraded in the last three years following three main objectives:
(1) establish a central national data node for life sciences for data management and analysis,
(2) establish a national infrastructure for obtaining high-density
data with high-throughput laboratory technologies (genomic technologies) and
(3) upgrade a center for education and training in life sciences.
The RI supports several experimental workflows with optimised steps, e.g. sample preparation, performance of laboratory work and bioinformatics analysis. The data generated with these workflows comply with the open science rules and the FAIR principles (Findable, Accessible, Interoperable, Reusable). There are already many ELIXIR-SI tools and services available that run on an ELIXIR-SI high performance computing (HPC) cluster and support common authentication and authorization infrastructure (AAI). More information about the ELIXIR-SI node and RI can be found on the web site: https://elixir-slovenia.org.

TE1

Do Fungal And Bacterial Communities In Ascocarps Shape The Arome Of True Truffles

Tine Grebenc[1], Nataša Šibanc[1], Aleksander Mahnič[2], Lidija Strojnik[3], Nives Ogrinc[3], Cene Gostinčar[4]

[1]Department of forest physiology and genetics, Slovenian Forestry Institute, Ljubljana, Slovenia. [2]National Laboratory for Health, Environment and Food, NLZOH, Maribor, Slovenia. [3]Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia. [4]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Truffles are the fruiting bodies (ascocarps) of fungi belonging to the genus Tuber that are fruiting in the soil and are best known for their aromas. Besides truffles ascocarps' produced aromatic volatiles, associated bacteria and yeast are also recognized to contribute significantly to the truffle. After extensive studies of truffles aromas in Europe (Strojnik et al. 2020, Šiškovič et al. 2021) and recent Tuber aestivum and T. magnatum whole genomes population re-sequencing, we aimed to analyze and correlate the outcome of aroma analysis with the bacterial and fungal communities on surface and within ascocarps of the same truffle ascocarps. Both, the bacterial and the fungal associated communities were further assessed with site and ecological characteristics of each truffle genotype. Results of the preliminary analysis and statistical assessment of truffle genomes diversity and associated bacterial and fungal communities will be presented.

Arbuscular Mycorrhizal Fungi – Ancient Organisms That Live at the Interface Between Plant Roots and Soils

Irena Maček[1], Nataša Šibanc[2], Dave Clark[3], Christoph Mueller[4],Thorunn Helgason[5,6], Alex J. Dumbrell[3]

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia [2]Department of Forest Physiology and Genetics, Slovenian Forestry Institute, Slovenia [3]School of Life Sciences, University of Essex, UK [4]Department of Plant Ecology, Justus-Liebig University Giessen, Germany [5]Department of Biology, University of York, UK [6]School of Biological Sciences, University of Edinburgh, UK

Arbuscular mycorrhiza is considered one of the most common symbioses in terrestrial ecosystems, with arbuscular mycorrhizal (AM) fungi living on the interface between roots and rhizosphere of more than 2/3 of plant species. AM fungi are thus an important component of the plant root microbiome, but due to their filamentous nature and dual mode of life, they can also be considered part of the soil microbiome. This means that these organisms have evolved in the past under the selection pressure of both environments: the direct pressure of abiotic factors in the soil and the pressure resulting from their biotrophic nature, since AM fungi are completely dependent on their host plants as a carbon source. We have used molecular tools to characterise AM fungal communities in a number of different environments that incorporate both biotic (plant-related) and abiotic (soil-related) factors that influence AM fungal community composition in plant roots. These systems include long-term FACE (Free Air CO2 Enrichment Experiment) with elevated atmospheric CO2 and extreme ecosystems (mofettes or natural CO2 springs) with geological CO2 exhalations inducing soil hypoxia. Comparative data from these studies on the composition of AM fungal communities as part of the soil and plant microbiomes will be presented identifying the most important drivers of AM fungal community composition in specific ecosystems.

TH1

Differences in gut microbiomes of lean and fat mice and changes related to nutritional shifts

Lipoglavšek Luka[1], Fanedl Lijana[1], Accetto Tomaž[1], Skulj Katja[2], Horvat Simon[2] and Avguštin Gorazd[1]

[1]Department of Microbiology and Biotechnology, Chair of Microbial Diversity, Microbiomics and Biotechnology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia. [2]Department of Animal Science, Chair of Genetics, Animal Biotechnology and Immunology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia.

The gut microbiome was proposed as an important factor in obesity very early in the era of microbiomics. Since then, a number of studies have been published in which authors have either confirmed or attempted to refute the original 2005 observation. The situation is still not resolved, due in part to the technologies used, the limitations of the animal models used, and, of course, the limitations of the experiments performed in humans. Below we describe the differences in the gut microbiome of polygenic mouse lines developed in a long-term selection experiment on high and low body fat content that has served as a unique animal model in many obesity/leanness studies over the past three decades. Two groups of Fat and Lean mouse lines, each with six animals, were subjected to dietary changes with low-fat and high-fat diets (the animals were bred in a seven-week dietary change regime), while the control groups of Fat and Lean mice (also each with six animals) were fed standard maintenance diets throughout the experiment. Animals were housed in pairs, but fecal samples were collected from individual animals at specific time points at the end of each feeding phase. The weight of the animals was monitored throughout the experiment. The short-chain fatty acids in the feces were determined and total microbial DNA was isolated from the fecal samples. The 16S rRNA V3 and V4 regions were sequenced using Illumina 2x250 bp technology, obtaining an average of approximately 50,000 sequence reads per sample. The differences between the microbiomes of Fat and Lean mice were detected suggesting the genotype effect. Moreover, we identified the differences in microbiome shifts following nutritional interventions, indicating a close relationship between the structure of a gut microbiome and diet composition.

Gut microbiome and systemic therapy in oncology

Martina Reberšek

Department of Medical Oncology, Institute of Oncology Ljubljana, Slovenia, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Gut microbiome has specific function in immunomodulation, host nutrient and drug metabolism, maintenance of structural integrity of the gut mucosal barrier and protection against pathogens. For healthy gut microbiome in eubiosis diversity of bacteria in the gut microbiota, a balance between proinflammatory and anti-inflammatory cytokines a balance between immune cells and IgA secretion and an intact and healthy mucosal barrier and mucus layer are very important. In dysbiosis different factors and chronic diseases interrupt this balance. The gut microbiome is also linked to development in different cancers, predominately of gastrointestinal cancers, and most notably of colorectal cancer (CRC). The CRC microbiota has a different composition of strains of bacteria than a healthy gut microbiome, and it includes strains individually linked to CRC. During the development of cancer, a complex interaction is established among the gut microbiome, tumour microbiome and immune system. Furthermore, the tumour microbiome has a negative impact on the gut microbiome, causing poor local and systemic responses of the host immune system as well as limited efficacy of systemic cancer treatment especially of immunotherapy with immune checkpoint inhibitors (ICIs). Different translational and clinical trial were done in melanoma, lung and kidney cancer to confirm that gut microbiota (GM) modulate the response to ICIs and the composition of GM is also associated to autoimmune adverse effects of ICIs and their severity. In melanoma patients it was shown that composition of GM in ICIs responders was different from GM in ICIs non-responders. In recent years manipulating the GM of cancer patients including dietary modification as most important, probiotics and faecal microbiota transfer is becoming of special interest and in development. In the future new generation of personalised cancer therapies will focus not only on molecular and phenotypic heterogeneity of different tumours but also on their specific microbiota and unique patient microbiota.

TH3

Changes in the microbiome along the invasion range of a successful freshwater invader

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Invasive alien species (IAS) drive biodiversity loss and impair ecosystem services worldwide. Increasing evidence shows the important role of microbiome in biological invasions. Both microbes in the novel environment and microbes carried by IAS, especially those pathogenic, can affect host's physiology, immune status, health and fitness. We have analyzed virome, bacteriome and mycobiome of one of the most successful freshwater invaders, the signal crayfish, Pacifastacus leniusculus. Using a metagenomic approach, we analyzed differences in microbiome of different tissues (exoskeleton, hemolymph, hepatopancreas, intestine) along invasion range of the signal crayfish. We compared the microbiome between individuals at the newly established populations (invasion fronts) of low crayfish density and long-established populations (invasion cores) of high crayfish density. Also, we studied differences in microbiome along different micro-environments, i.e., between individuals from upstream and downstream river segments. Surprisingly, we did not establish the presence of known crayfish pathogens in none of the analysed microbiome components (virome, bacteriome, mycobiome), but have recorded a high diversity of many previously unreported taxa for freshwater crayfish. We established differences in crayfish microbiomes both along invasion range and different river segments, indicating that both dispersal process and the environment shape microbiome structure and composition. Our findings offer an insight into microbiome changes during dispersal of a successful invader and present a baseline for assessment of their contribution to invader's overall health and its further invasion success.

Microbiome of Slovenian cultural heritage objects

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Deterioration of cultural heritage objects is a major concern for every generation in order to preserve them for the future. Among the different types of damage, biological damage (biodeterioration) is the most destructive. Microbial communities can attack materials of organic and inorganic origin. They colonize surfaces through air and dust deposition, precipitation, and human activities and thrive when conditions are suitable for their growth. In particular, fungi play an important role due to their widespread distribution, secretion of various enzymes and acids that subsequently cause degradation of a variety of materials, dissolution of minerals and biomineralization, production of various pigments that discolor objects, mechanical damage due to expansive growth, etc. The most important trait for successful colonization of indoor artworks by fungi is their ability to grow at low water activity or low relative humidity even under recommended microclimatic conditions. Understanding and managing biological damage requires multidisciplinary approaches and the use of state-of-the-art molecular techniques in combination with classical techniques such as microscopy, application of culturing techniques, and in vitro laboratory testing. Over the past decade, fruitful collaborations have been established with Slovenian and international communities dealing with cultural heritage. Microbiomes (fungi, bacteria) of numerous art paintings from churches and museums, stone monuments, archival material, and murals at archeological sites have been studied, some with 454 pyrosequencing, others with Illumina amplicon sequencing of targeted genes (ITS2 for fungi, partial 16S rRNA for bacteria) that allow community identification. Numerous obstacles had to be overcome, from non-destructive sampling to distinguishing between active cells. In parallel, the use of culturable methods enabled isolation of key players in pure cultures and in vitro assays, as well as genome and transcriptome sequencing (in progress). All this will contribute to the understanding of overgrowth and deterioration of cultural heritage items, and hopefully aid in suggesting solutions.

TEg2

Cold plasma treatment alters fungal community of buckwheat grains

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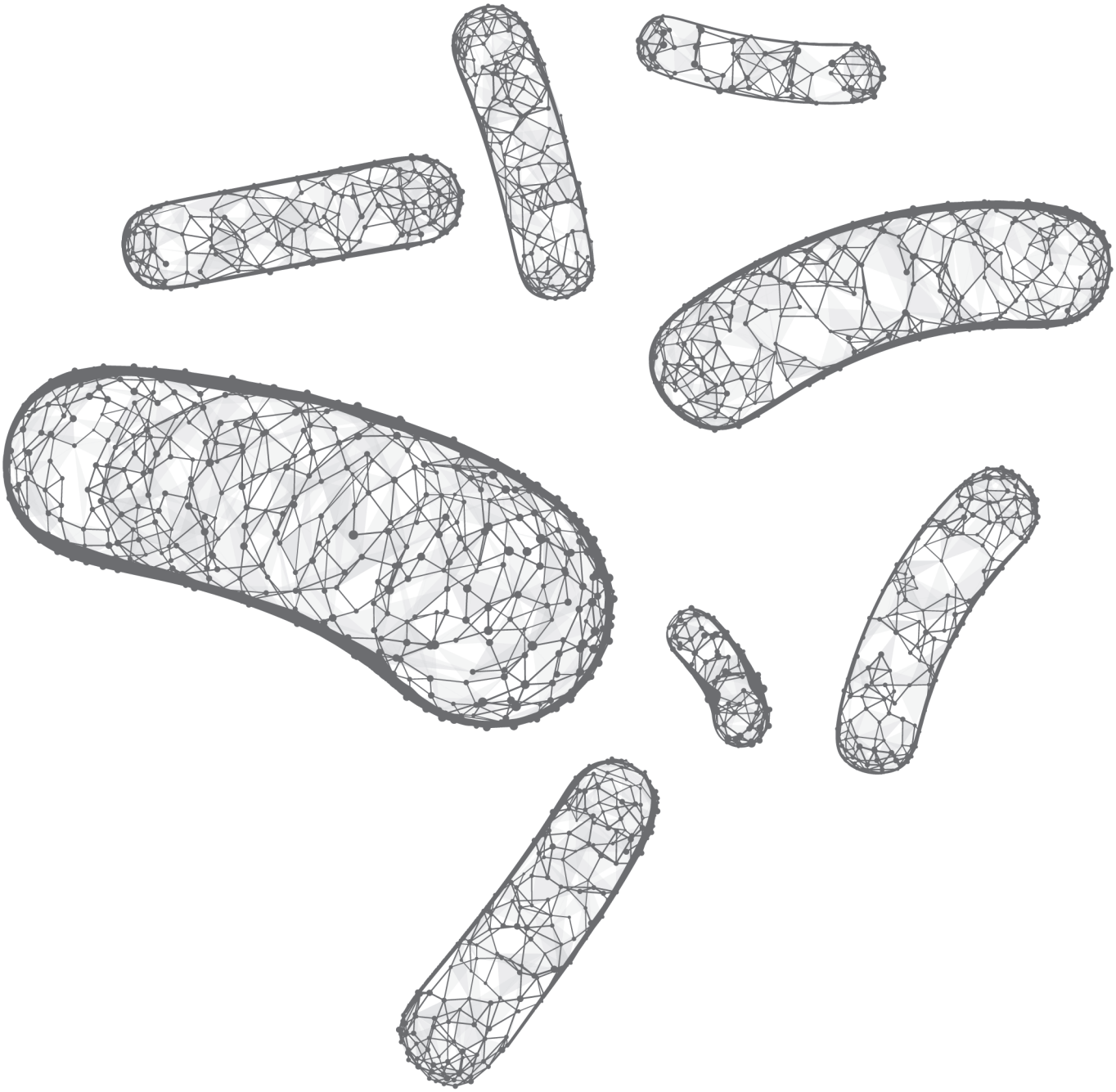
Fungal contamination is one of the main concerns in cereal grain production that can occur at all stages, from preharvest to postharvest processes and during storage. Fungicides and other artificial chemicals are still the most widely used to prevent fungal infections. However, they leave residues and negatively impact our environment and human health. Therefore, new alternative approaches for grain treatment are needed. Cold plasma (CP) treatment offers potential as a green technology for surface decontamination of seeds and grains without using chemicals. CP is a partially ionised gas, the fourth state of matter, that can be generated by applying thermal or electrical energy to a gas. It consists of free electrons, atoms, molecules, ions, radicals, other reactive species and ultraviolet and vacuum ultraviolet radiation. All these species give CP unique properties and also antimicrobial activity. We investigated the effect of powerful low-pressure radio frequency CP setup in direct ("glow") and indirect ("afterglow") operational modes on the fungal community structure of buckwheat grains. The decontamination was more effective in glow treatment, as the initial contamination reduced to less than 30% in common buckwheat (CB) and 10% in Tartary buckwheat (TB) grains after the 60-second treatment. CP also affected fungal diversity as only a few genera (mostly Alternaria, Didymella and Epicoccum) still persisted after the glow treatment. Afterglow treatment resulted in a lower reduction of initial fungal contamination (up to 30% in CB and up to 50% in TB) and had less impact on fungal diversity. However, the glow treatment also drastically reduced the germination of buckwheat grains, making it suitable only as a postharvest treatment and not for further sowing.

ABSTRACTS OF POSTER PRESENTATIONS



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P1

In Search Of Microbial Bioindicators Of Physico-Chemical Properties

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The composition of aquatic microbial communities can vary according to the physico-chemical parameters of the freshwater, including nutrients, major ions and trace elements. As they respond to changes in the environment, it is now evident that microbial communities can complement traditional taxa such as algae and macroinvertebrates as bioindicators of water quality. Here, we aimed to better understand the effects of physico-chemical parameters of the water, including trace elements, on microbial community structure and the occurrence of putative bioindicator taxa. We collected a variety of surface water samples throughout Croatia (n = 35) and analysed their physico-chemical properties, including the concentration of trace elements determined by the high-resolution inductively coupled plasma mass spectrometry method (HR-ICP-MS). In addition, total environmental DNA (eDNA) was extracted from the water samples and used to analyse the composition of the microbiome by high-throughput sequencing of the 16S rRNA marker gene. The partial least squares regression (PLS-R) modelling results identified a number of microbial taxa that were positively correlated with some of the water parameters. For example, some microbial taxa belonging to the Proteobacteria phylum were positively correlated with ion content in water (i.e. SO42-, Ca2+, Cl-, Na+ and EC), while some taxa from the Firmicutes phylum were correlated with nutrient content (ammonium and total phosphorus). In addition, uranium was the most important trace element influencing the microbial communities in the water and was positively correlated with the occurrence of many proteobacterial and actinobacterial taxa. In summary, the results obtained will help in the development of protocols for eDNA-based biological assessment of water quality.

P2

Investigation of Slovenian irrigation waters' viromes with a focus on plant viruses

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High-throughput sequencing has enabled in-depth analysis of the virome of many ecosystem components, including various aquatic systems. We undertook a metagenomics study of irrigation water from selected Slovenian tomato farms. Samples were first concentrated using monolithic chromatography, RNA extracted using a Trizol-based protocol, and randomly preamplified for sequencing. Sequencing was performed using the Illumina MiSeq platform, and a custom bioinformatics analysis pipeline was used for data analysis. The main result of the study was the detection of nucleic acids from several known and putative new plant virus species. The source of irrigation water had an impact on the diversity and number of virus species identified. The most prevalent viruses belonged to the genera Tobamovirus and Tombusvirus, which are known to contain viruses with environmentally stable virions. Our findings suggest that information about plant viruses circulating in the environment can be obtained by studying the virome of water samples, which include detection of new, previously unknown viruses classified in viral genera, which contain economically important pathogens. This approach may serve as the foundation for developing water-based epidemiology approaches for the detection of plant virus outbreaks in crops and surveillance of emerging pathogens.

P3

How Is Flooding Disturbance, As A Consequence Of Climate Change, Affecting Soil Microbiome

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As a consequence of climate change, flooding frequency is predicted to increase during the next decades, calling for a better understanding of its impacts on soil ecosystems and for developing strategies to mitigate potential damage. This is especially important for vulnerable agricultural sector, where floods can reduce crops productivity and quality, and consequently food security. Even though successful plant growth and resistance is directly connected to healthy interaction existing between plant and its soil microbiome, there is still little knowledge about how soil microbiome structure and functions is affected by the flooding disturbance. Project entitled "Potential of the rhizosphere microbiome in the adaptation of agriculture to climate change (PERSPIRE)", funded by the EU Regional Development Fund, is focused on the effects of floods on both development of plants as well as the response of its soil microbiome. Project is based on the experiment conducted in the greenhouse-controlled conditions (16 h day/8 h night; 25 ˚C per day/20 ˚C per night; 60-70% relative humidity), with the cabbage (Brassica oleracea var. capitata f. alba) used as a model plant. Plants (triplicate trials) were subjected to either one or two long-term flooding events (7 days duration of each flood) at different stages of development. At different time points (day 0, after flooding and after recovery period) whole plant as well as soil was removed from the pots and subsamples were taken for culture-based and molecular-based microbiome analyses with the aim to determine flooding disturbance effect on the structure of both total soil microbiome as well as on the population of plant growth promoting bacteria (PGPB). This knowledge will contribute to a better understanding of the impacts of flood stress on soil microbial communities with the final aim to propose new strategies that can help in crop adaptation to a changing climate future scenario.

P4

Aquatic Microbiome Of The Northernmost Mediterranean Salterns

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Man-made marine salterns consist of a multi-pond system in which water gradually evaporates until precipitation of halite, the main product of the activity. After the salt is harvested, the salt pans remain with the bitterns. The aim of our study was to describe the microbial diversity (archaea, bacteria, fungi and other eukaryotes) in brines of different concentrations and in bitters from the Sečovlje salterns (Slovenia), the northernmost Mediterraneas saltworks. We used a combination of metagenomics (amplicon sequencing on Illumina platform) and culture-based methods to study the microbiota in the water of salterns. From brines of varying salinity, we isolated bacterial strains belonging to the genera Bacillus, Chromohalobacter, Cobetia, Halomonas, Larsenimonas, Salicola, Salinicola, and Staphylococcus, as well as archaea from the genera Halococcus, Halorubrum, and Halovivax. Using amplicon sequencing of the 3rd and 4th variable regions of 16S rDNA, we found that the predominant bacterial genera in the brine and bittern of the crystallization ponds were Salinibacter and Salinivenus, while the predominant archaeal genus was Halorubrum. The majority of fungal isolates from brine and bitters belonged to Ascomycota, genera Cladosporium, Aspergillus, and Penicillium; a few were basidiomycetes, mostly from genus Wallemia. Black yeast-like fungi (Dothideales) and non-melanized asco- and basidiomycete yeasts were also present. Amplicon sequencing of the ITS2 region allowed us to detect 73 different fungal genera. Using 18S rDNA amplicon analysis, we also identified other eukaryotes, most notably ciliates from the genera Fabrea and Eutintinnus, from Myzozoa genus Euduboscquella, and green algae from the order Chlamydomonadales. With increasing salinity, the alpha diversity of bacteria, archaea and other eukaryotes decreased, while the alpha diversity of fungi was lowest at moderate salinity (10 - 20 °Bé). By analyzing the results on the presence of halotolerant/halophilic organisms in the Sečovlje salterns, we demonstrated the necessity of complementarity of cultivation- and metagenomic-based approaches.

P5

Metagenomic Insight Into Microbiomes Of Two Pristine Karst Aquifers (In Slovenia): So Much Water Underground But Where Are The Microbes?

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About ninety-five percent of global liquid freshwater is stored in the terrestrial subsurface, making it the largest terrestrial freshwater biome. Despite the importance of this water source for humanity, these systems are largely understudied from the microbiological point of view. In Slovenia, about half of the population depends on groundwater for its domestic needs. The quality of this water source and the putative presence of pathogens are not properly monitored. Accordingly, very little is known about the composition and the structure of its native prokaryotic communities. We analyzed a time-series of 14 months of sampling from two pristine karst groundwater reservoirs. A number of physical and chemical parameters (n=21) were monitored and microbial cells were size fractionated into >5 um, >0.45 um and >0.1 um fractions. Large volumes (100 l / filter) were filtered to obtain measurable quantities of DNA for metagenomic sequencing. Here we present the basic environmental characteristics, physicochemical parameters coupled to shotgun metagenomic data obtained from various size fractions of microbial communities and provide basis for massive assemblies of available cave shotgun sequencing to metagenome assembled genomes in the future. These results are not relevant only for the understanding of the microbial dynamics in the karst aquifers, but also as high-through put tools for modern water management in attempt to minimize the pressure on underground ecosystems and protect them as drinking water resources for the future.

P6

Identifying Potential Host-Specific Indicators Of Fecal Contamination Using Amplicon Sequencing

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Precise differentiation of the sources of fecal contamination in recreational and drinking waters is important for planning water remediation strategies and assessing human health risks. As gut microbiota composition is species specific, microbial source tracking (MST) methods can be used to differentiate between sources of fecal contamination by detecting host-associated markers in contaminated water samples. Numerous human-associated MST markers have been previously identified and evaluated. However, additional research is needed for the development of reliable markers of animal fecal pollution. The aim of this study is to identify potential MST markers for determining the sources of fecal pollution deriving from domestic and wild animals common in Central Europe. Fecal samples (n = 287) from 33 different animal species and humans were subjected to 16S rRNA gene (V3-V4 hypervariable region) sequencing and analysis of operational taxonomic units (OTU) and zero-radius OTU (ZOTU). For animal taxa with five or more samples (n = 18), the number of OTUs and ZOTUs with 100 % specificity, sensitivity higher than 90 % and abundance higher than 0.1 % was calculated and taxonomy was assigned to each OTU and ZOTU matching our criteria. A variety of OTUs and ZOTUs matching our specificity, sensitivity and abundance criteria were detected and could potentially be developed into molecular markers for MST. The majority of them were found in sheep, pigs, cats and wild rodents (nutria and mice). More than 85% of host associated OTUs and ZOTUs found belong to phyla Bacteroidota (Bacteroidales) or Bacillota (Firmicutes).

P7

First Glimpse Into The Tomato And Pepper Viromes Of Croatia

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Tomato is an important crop, hosting at least 312 viruses, satellites or viroids. Within the Croatian-Slovenian project "Nanopore high throughput sequencing for the resolution of problems in the plant pathogen epidemiology and diagnostics", main sampling sites were greenhouses in the continental Croatia (Vidovec, Sedlarica). In 2021, tomatoes from Dalmatia (Turanj) and neighboring symptomatic peppers were also collected. Leaves were used for lateral flow tests (LFT) and nucleic acids extraction. For nanopore high throughput sequencing (HTS) library preparation, representative tomato DNase treated CTAB detergent extracts were pooled, rRNA depleted and polyA tailed. Based on HTS and LFT results, tomato and pepper samples were individually tested by reverse transcription polymerase chain reaction (RT-PCR) with specific primers. Preliminary results showed coinfection with potato virus S (PVS, belonging to the PVSO strain) and potato virus Y (PVY, clustering with PVY-NTN strains) in one tomato from Vidovec. PVY was also detected in other tomato samples from Vidovec and Sedlarica. A severely diseased tomato from Turanj harbored only tomato spotted wilt virus (TSWV). Peppers were either infected by cucumber mosaic virus (CMV) (two from Vidovec) or by potyviruses and a pospiviroid (one from Sedlarica and Vidovec). A pospiviroid amplicon was obtained for one of the Sedlarica PVY+ tomatoes. Partial sequences suggest Citrus exocortis viroid (CEVd) is distributed in tomatoes and peppers but this needs to be confirmed. This first glimpse into the Croatian tomato and pepper viromes suggests PVY was predominant in the continental tomatoes with a virus or a viroid occasionally in coinfections. A few peppers tested so far had viromes constituting of CMV, potyviruses and pospiviroids.

P8

Structure And Diversity Of The Microbial Communities Of Sessile Stygobionts In The Dinaric Karst

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The Dinaric karst underground is inhabited by many endemic and relict taxa, including the only known cave-adapted genus of bivalve mollusks from the genus Congeria. However, the microbial communities of the Dinaric karst underground have not been sufficiently studied and there are no data on the microbiome of cave invertebrates. In this study, the microbial communities of the stygobionts of the Dinaric karst, including all three Dinaric cave mussel species (Congeria jalzici, C. kusceri and C. mulaomerovici) and the Dinaric cave tubeworm (Marifugia cavatica), are described for the first time. Tissues from mussels and tubeworms, biofilm from mussel shells and water were sampled at four sites in Croatia and Bosnia and Herzegovina. Bacterial taxa were identified by 16S rRNA gene high-throughput sequencing. A total of 43 bacterial phyla, of which Proteobacteria were the most abundant, and 373 families were detected. Significant differences in microbial community composition were found between the different sample types, i.e. tissues of mussels and tubeworms, biofilm of mussel shells and water. The microbial communities of tubeworms differed significantly by site, suggesting that site-specific environmental factors might influence the host and its microbial symbionts. In contrast, the microbial communities of bivalves did not differ significantly by species or by site, probably due to the small sample size. In conclusion, this study provides the first data on the microbial communities of sessile filtrators in the Dinaric karst underground and is therefore a basis for further research on food webs and host-microbe interactions in the Dinaric karst underground.

P9

The Mycobiome Of A Successful Crayfish Invader: An Overlooked Component In Biological Invasions

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Microbiome affects various interactions between the invader and its novel environment during the process of biological invasion. In turn, dispersal process and characteristics of the novel environment may affect the composition of invader's microbiome, directly and indirectly affecting its fitness and invasion success. So far, the majority of studies focus on bacteriome, insufficiently addressing other components of microbiome, such as mycobiome. Microbial fungi are among the most damaging pathogens in freshwater crayfish populations, with both native and invasive crayfish species being susceptible to fungal colonization and possible infection. Using ITS rRNA amplicon sequencing, we have analyzed the mycobiome of a successful invader in Europe, the signal crayfish. We explored the mycobiome of four types of crayfish samples (exoskeletal biofilm, hemolymph, hepatopancreas, intestine), compared them to environmental samples (water, sediment), and examined differences in fungal biodiversity and abundance between upstream and downstream segments of the signal crayfish' invasion range in the Korana River, Croatia. A small number of amplicon sequence variants was obtained from hemolymph and hepatopancreas samples, pointing to small fungal abundance. Thus, only exoskeleton, intestine, sediment and water samples were analyzed further. Significant differences were recorded between their mycobiomes, confirming their uniqueness. Environmental samples showed higher biodiversity than crayfish-associated samples. Intestinal mycobiome showed significantly lower richness compared to other three mycobiomes. Sediment and exoskeletal mycobiome differed significantly between different river segments, indicating that environment (i.e. sediment mycobiome) at least partly shapes the exoskeletal mycobiome of crayfish. Intestinal mycobiome showed no differences between river segments, which indicates that this internal organ's mycobiome remains more stable despite environmental changes. Our results present the first metagenomic data on crayfish-associated fungal communities across different tissues, and offer a baseline for assessing how mycobiome contributes to species' overall health and further invasion success.

P10

How Does A Commercial Probiotic Preparation Affect The Cecal Microbiota In Broilers?

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Fecal contamination of poultry meat at slaughter is a common source of infection in foodborne campylobacteriosis and salmonellosis. Administration of probiotics is an alternative approach to reduce colonization of the broiler gut with bacterial pathogens. To this aim, we investigated the effect of a multispecies probiotic preparation on broiler cecal microbiota, focusing on Campylobacter jejuni and Salmonella Infantis. Two large food business operators (FBO) were included in the study to perform the field trial, which was repeated twice. The preparation was administered either into the water or into the feed, whereas the control group received no preparation. Cecal contents were collected for DNA extraction at different time intervals. Sequencing libraries were prepared according to the 16S Metagenomic Sequencing Library Preparation guide (Illumina), targeting the V3–V4 hypervariable region, and paired-end sequencing (2×300 bp) was performed on the MiSeq System (Illumina). Results showed that shifts in the composition of microbiota were largest at the early stages of the breeding cycle. In adult broilers, Firmicutes predominated and their richness increased with broiler age. In addition, unclassified amplicon sequence variants (ASVs) were detected and increased with age, indicating the presence of undescribed taxa in the broiler gut microbiota. The trial repetition and location (FBO) significantly affected the metagenome, whereas the effect of the probiotic preparation was small. When it was administered into the feed, the proportion of Salmonella-colonized broilers slightly decreased, but no effect was observed with probiotics administered into the water or regarding C. jejuni colonization. The latter correlated significantly with metagenome composition, especially with the reduced relative abundance of two ASVs that may act as C. jejuni antagonists. We can conclude that the tested commercial probiotic preparation has a negligible effect on reducing the colonization of broiler gut with Campylobacter and Salmonella in field conditions.

P11

Assembling Complete Genomes Of Potato Endophytic Microbes

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Endophytes encompass microbes, which for all or part of their lifetime colonize the internal of plant tissues. They can be commensals with no apparent effect on their host, beneficial plant growth-promoting microbes or also latent pathogens. Beneficial endophytes can directly promote plant growth through nitrogen fixation, synthesis of enzymes or peptides that provide nutrients, or by the production of phytohormones. On the other hand, they can protect the plant either directly, by antibiosis or competition for nutrients with the pathogens, or indirectly, by priming the plant's immune response. We isolated three microbes, two bacteria and one fungus, from tissue culture cultivated potato plants propagated in a sterile environment for several years without any visible signs of infection. Thus, it is reasonable to assume that they are endophytes tightly associated with potato plants. To precisely determine their phylogeny and get insight into their potential functions, we sequenced their genomes by a combination of Nanopore and Illumina sequencing. For bacteria, most contiguous genomes were obtained using trycycler, by combining the output of several long-read assemblers followed by Illumina reads polishing. For the fungus, a hybrid assembly using WENGAN produced the highest quality genome. Taxonomic analysis suggests that one of the isolates belongs to a novel species. We will present its gene repertoire, comparison to phylogenetically most similar bacteria and biochemical determination of some metabolic functions. Our assembled genomes facilitate hypothesis-driven functional characterisation of the isolated endophytes and the understanding of their interactions with the host plant.

P12

Bacteriobiota Of Olive Leaves With Olive Leaf Spot Disease Symptoms Compared To Asymptomatic Leaves

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Olive leaf spot, caused by the fungus Spilocaea oleagina, represents one of the most important diseases of olive trees. The study aimed to evaluate the microbiome in infected and asymptomatic leaves of the highly susceptible variety 'Istrska belica' and finally, to identify differentially abundant (DA) taxa which could act antagonistically on the pathogenic fungus or to provide information regarding correlations between core taxa. In total, 14 trees were randomly selected (7 trees for each leaf's health status) from the olive orchard near Bonini (Koper, Slovenia). A V4 region of 16S rRNA was amplified with 515F and 806R primers and sequenced on the Ion S5™ System. Reads were firstly analyzed with QIIME2 using denoise-pyro and classify-sklearn plugins. Taxonomic classification was performed based on Silva 138 99% OTUs from amplicon region specific pre-trained classifier and the Maaslin2 package was used for differential abundance analysis. Previously performed sequencing of the ITS1 region confirmed increased presence of S. oleagina (from 12.4 to 24.5%) whereas from 0.6 to 2.8% of reads belonged to S. oleagina in symptomless leaves. These data indicated possible infection of symptomless leaves during incubation period or that the inoculum was present on the leaf surface. A significant difference was observed for the alpha diversity Shannon index (Kruskal-Wallis test) as well as for beta diversity based on unweighted UniFrac distance (PERMANOVA test). Four genera were significantly overrepresented in infected leaves (Sphingomonas, Methylobacterium-Methylorubrum, Hymenobacter, and Mucilaginibacter). The first three genera were also the most highly represented among all DA genera based on average value across all samples (from 15 to 25%). Acinetobacter, Staphylococcus and Corynebacterium were underrepresented in infected leaves and on average represented from 1 to 5%. Other 14 DA genera, underrepresented in infected samples, were on average presented with less than 1% of reads among all samples.

P13

Using Plant Virology High Throughput Sequencing Data In Screening For Other Potential Plant Pathogens

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The Euphresco project Plant Health Bioinformatics Network (PHBN) initiated a community effort to re-analyze existing RNA-seq datasets intended for virus detection. High throughput sequencing (HTS), mainly RNA-seq of plant samples is often used by plant virologists searching for potential virus infections. Re-analyzing those datasets for the potential presence of non-viral pathogens or pests could be useful for wider phytopathological communities.
Eight datasets available at Agricultural Institute of Slovenia were analyzed by first mapping the data against a given rRNA database based on SILVA. The datasets came from different plant species (soybean, grapevine, garlic, and Rubus spp.) and only in three datasets plant virus sequences were confirmed. All the results were sent to ILVO for evaluation of the obtained results and four datasets were selected for more detailed analysis using direct taxonomic classification of the reads using Kraken2 against the complete Genbank non-redundant Nucleotide BLAST database. The resulting taxonomic classifications were checked for the presence of non-viral plant pests and pathogens and the plausibility of their presence was evaluated. The analyzed Rubus dataset showed a clear evidence of aphid infestation (Aphis gossypii, 3063 reads per million (rpm)), while one of the soybean datasets indicated a presence of mites (Tetranychus urticae, 345 rpm). In the two soybean datasets, traces of oomycete reads (Phytophthora spp.) were also found, at 295 and 276 rpm respectively. The garlic dataset contained reads attributed to Fusarium spp. (726 rpm). These results confirm that RNA-seq datasets used for plant virus detection can be used to find traces of other plant pathogens as well, although the exact species identification sometimes remains unclear. This work will hopefully improve and refine our knowledge on microbiome associated with viral infections in plants.

P14

On The Intersection Of Metagenomics And Culturing; The Rumen Case

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Metagenomics is immensely popular and currently engaged in solving many of the pressing questions in biosciences. Here, we focused on a simple and elementary problem, which seems solved, especially in benchmarks accompanying metagenomics-oriented algorithms and pipelines: how well do the metagenome assembled genomes (MAGs) really represent cultured bacterial strains and species? We used 516 Prevotellaceae MAGs from a major study on rumen microbiomes (10.1038/s41587-019-0202-3) and compared them to isolated ruminal Prevotella while isolating new strains. Using bioinformatic and culturing approaches, the main findings were:

- The MAGs frequently lack (>2 in 396/516) or have duplicated/triplicated (195/516) conserved marker genes commonly used for phylogenetic analysis and identification (ribosomal protein, elongation factor, RNA polymerase genes) in spite of high checkM scores. Fast classification of newly isolated Prevotella strains among existing MAGs using PCR was in practice severely hindered and genome sequencing had to be used to obtain reliable results.
- The missing/duplicated genes are found in clusters and have oligonucleotide usage significantly different from the rest of the genome. The prime example is a ribosomal protein gene cluster conserved in most bacteria. The reason for oligonucleotide usage departure was the codon bias (optimization) for efficient translation.
- The ribosomal clusters of cultured Prevotella cannot be differentiated based on their tetranucleotide content rendering the existing algorithms of metagenome bin sorting without the main sorting criterium.
- We devised an approach for removal of false positive ribosomal cluster contigs from MAGs.
- The MAGs corresponding to known Prevotella capture well CAZyme content and are largely syntenic.
- We isolated a Prevotella strain from sheep rumen corresponding to MAG from the above-mentioned study. The MAG contained six duplicated and four triplicate ribosomal genes and lacked a 205 kbp plasmid of the isolated strain. Though the latter may be natural variation, plasmids and phages are predicted to suffer same missorting as ribosomal genes.

P15

Searching For Consensus In Black Soldier Fly Microbiomes: A Cross-Study Analysis

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Hermetia illucens has emerged as the most versatile species for industrial insect rearing, as its larvae efficiently convert organic wastes into insect biomass, thereby forming a sustainable resource for value-added commodities such as feed, chitosan, biodiesel, and fertilizer. The larvae's affinity for spoiled organics and their ability to exploit wastes as their natural habitat inherently emphasizes the importance of microbes in their lifecycle. Thus, a multitude of studies on the gut microbiome of black soldier fly larvae have been published over the past decade, employing a vast diversity of sample preparation protocols, sequencing strategies, data analysis pipelines, and databases. However, the lack of a best practice approach hampers comparability across studies, making the interpretation of collective results challenging and afflicted with bias. Here, we present a workflow for selecting, merging, and analyzing datasets of published studies using high-performance computing built on Human Microbiome Project best practices. For this collection of more than 20 studies from 18 countries, we re-calculated abundance and taxonomy data at genus, OTU, and ASV levels with subsets of various sequencing depths. This standardized analysis unveiled cross-study patterns such as a prevalent and recurring group of Genera including Enterococcus, Actinomyces, Providencia, Dysgonomonas, and Morganella. These results provide insights into microbe-host interactions of Hermetia illucens and give indications on what makes up a healthy larval gut microbiome. Moreover, the results emphasize the need for standardization to ensure comparability and increase significance for future studies.

P16

"Born To Run": Insights Into Human Intestinal Microbiota And Metabolome Dynamics Of Normoxic And Hypoxic Bedrest Studies

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Separate and joint effects of physical inactivity, a reduction in gravity due to the horizontal immobilization (hydrostatic pressure) and the partial reduction of oxygen saturation (hypoxia) on the human intestinal microbiota and intestinal metabolites in healthy volunteers were investigated under medical supervision in controlled conditions for 21 days. Samples were obtained within EU PlanHab project (Planetary Habitat Simulation project EU FP7-space; http://www.planhab.com/; PI: Igor Mekjavić, IJS: Jozef Stefan Institute, Ljubljana). Stool samples were collected during run-in period (days -5 and -1 before the onset of experiments) and days 3, 10, 18 and 21 of the three experimental settings ((i) normoxic bedrest, (ii) hypoxic bedrest and (iii) hypoxic ambulatory). Deep sequencing using paired-end MiSeq approach was used for fine scale characterization and identification of key microbial groups and their functional role responding to environmental perturbation. 1H-NMR was used for targeted metabolomics profiling. Existing human physiological data were compiled for the same days of experiments. The influence of food intake (quantity and nutrient composition) on microbial composition was also analyzed and used as control for comparison between baseline data collections and post experimental variants. Significant changes in physiology coincided with changes in intestinal environment but preceded or took place in absence of significant changes in bacterial community structure. Highly responsive Bacteroides with inflammagenic characteristics and genes involved in iron acquisition, cell wall, capsule, virulence and mucin degradation were enriched in physiologically most detrimental variant. The first hierarchical model linking initial body deconditioning and microbiome in response to the acute inactivity was derived. Analyses are being extended to thousands of samples in the Human Microbiome Project in 2022.

P17

Mago Tool Assembled Genomes From The Shotgun Sequences Of Fecal Samples From The Planhab Project

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We studied the dynamics and the diversity of the gut microbiome in response to reduced physical activity within the EU FP7 PlanHab project experiments. Our open-source-software package Metagenome-Assembled Genomes Orchestra (MAGO) was used for metagenome assembly, binning, bin improvement, bin quality control and bin annotation of reads obtained with shotgun seguencing (Illumina Inc., USA) from the fecal samples of the participants from the PlanHab study (MG-RAST). MAGO enables quality control and processing with FastQC and fastp and then makes use the three most popular assemblers (metaSPAdes, megaHIT, idba-ud) and six binners (MaxBin, MetaBat, CONCOCT, BinSanity, BinSanity-wf, BinSanity-lc). The resulting bins are improved by DAS tool and checked with CheckM (percentage of completeness and contamination). MAGO also enables building maximum likelihood protein trees with ezTree, prokaryotic genome annotation with Prokka, pan- and core-genome analysis with Roary and average nucleotide identity with FastANI. As a simple analysis, according to Tetra Correlation Score (TCS) on JSpeciesWS we assembled 21 different strains of Streptococcus thermophilus, 8 different strains of Haemophilus parainfluanze, 3 different strains of Eubacterium siraeum, Phascolarctobacterium succinatutens and Ruminococcus bicirculans, Eubacterium eligens, next to 4 different strains of Eubacterium rectale. All newly assembled high quality MAGs assembled so far in this study (n=40) were classified as part of the human gut microbiota. This study shows the power of MAGO for reconstruction of metagenome-assembled genomes from raw sequence data and represents the first case of direct reconstruction from microbial metagenomes of Slovenian participants. Further analyses are being conducted on expanded human gut shot-gun data collections.

P18

Efficiency Of Fecal Microbiome Data Layers In Classification Of Depression

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Depression is the most common mental disorder in the world but despite this, the methods used for its diagnosis are still problematic and inadequate. Diagnostic classification could be improved with non-invasive, quantitative tests based on biomarkers. While numerous biomarkers have already been researched, a potentially promising target has remained overlooked as systems biology approaches were not utilized. Gut microbiome (GM) is one of the most important parts of the gut-brain axis, a bidirectional pathway that appears to be dysfunctional in depression, GM represents information rich subsystem, but no single biomarker could be identified so far. To explore the efficiency of fecal microbiome data layers in classification of depression a metagenomics subset of the Flemish Gut Flora Project was acquired (n=150) including metadata (age, sex, BMI, BSS, RAND), 80 with depression and 70 healthy controls. The sequencing data was pre-processed with metaBakery implementation of bioBakery (KneadData, HUMAnN3; MetaPhlAn3;mothur; databases) at HPC Vega, which extracted information layers of taxonomy (Bacteria, Archaea, Fungi, Protozoa, DNA Viruses), diversity, functional genes, enzyme reactions, metabolic pathways, and intestinal metabolites. The importance of features within these layers of information for differentiation between healthy and diseased individuals and those with depression were explored and utilized to build, optimize and validate classification models in Orange and JADBio. While results from individual GM layers were promising, the classification model utilizing assembled information from all GM layers yielded >90% classification accuracy. This clearly shows the benefits of using gut microbiome information in classification of psychiatric disorders.

P19

Escherichia coli from Gut Microbiota Affect Human Circadian Clock

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Dysbiosis of the gut microbiota can lead to an altered circadian clock of the mammalian host. The aim of this study was to reveal whether different Escherichia coli strains (BJ17 and BJ23) from the gut microbiota of healthy humans can affect the expression of human circadian clock genes in an in vitro model. The E. coli strains were grown in co-culture with human HepG2 hepatoma cells and expression of the firefly luciferase reporter under the control of Period2 promoter was followed. RNA from HepG2 cells was extracted by Trizol reagent, treated with DNase I, transcribed to cDNA and Q-PCR analysis for expression of central genes of the circadian clock was performed. Microsoft Excel was used for calculation of the mean ± SD and to perform the linear regression analysis. For comparison analysis of circadian genes expression GraphPad PRISM v6.01 (GraphPad Software) was used (Sidak's comparison test). Rhythmicity analysis of observed circadian genes was performed with a single-component cosinor model for which a 24-hour period was presumed. HepG2 cells with stably integrated firefly luciferase reporter under control of the Period2 promoter were inoculated with either BJ17 or BJ23 E. coli strain and their co-culture was followed for 25 hours with the Lumicycle apparatus. After 4 hours the signal of the measured luminescence in co-cultures decreased, while the luminescence signal of the control, cells without bacteria, was still increasing. Addition of bacteria enhanced the circadian behavior of CLOCK and BMAL1. The statistically significant change of the amplitude, compared to controls, was observed for HepG2 genes PER1 (decrease) and PER2 (increase) grown with E. coli BJ17. The change in the phase of gene expression (phase shift) was observed only for PER3, again grown with E. coli BJ17. Thus, individual E. coli strains can have a different impact on the host circadian clock genes.

P20

Evaluation Of Targeted High-Throughput Sequencing For The Determination Of Lung Microbiome

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Targeted high-throughput sequencing (HTS) is an important new tool that gives us insight into the diversity of the microbiome and can provide us with important new insights into patient health. Therefore, it is important that we have confidence in the microbiome results we obtain with targeted HTS. As with any new method, there are some obstacles we must overcome before we can truly trust the results. In our study, we investigated the impact of the DNA extraction method and library preparation kit, as well as the repeatability of targeted HTS results. We used sputum samples collected at University Clinic Golnik, Slovenia. DNA was extracted using three different methods: an in-house cetrimonium bromide (CTAB) solution-based extraction; and two commercially available kits for solid phase extraction, one based on magnetic beads and the other on silica membranes. Microbiomes were determined by sequencing the 16S rRNA region using the IonTorrent platform. To prepare the libraries for targeted HTS, we used Ion AmpliSeq™ Pan-Bacterial Research Panel and 16S™ Metagenomics Kit. We found that the DNA extraction method had a significant impact on microbiome richness, diversity, composition, and percentage of gram-positive bacteria. The microbiome in DNA samples extracted using the silica membrane method had (on average), the highest richness and diversity and the most repeatable results. The library preparation kit also showed effects on the richness, diversity, and composition of the microbiome. The 16S™ Metagenomics Kit provided higher richness and diversity. Microbiome results obtained with targeted HTS were repeatable when DNA quality was sufficient, and sequencing generated a sufficient number of reads. Targeted HTS proved to be a reliable method for determining the microbiome in sputum samples. However, care must be taken when planning experiments to determine which DNA extraction method and library preparation kit to use.

P21

Virome Of The Invasive Signal Crayfish And Its Variations Along The Host's Invasion Range

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Crayfish are keystone species of freshwater ecosystems and successful invasive species, however, their pathogens, including viruses, remain understudied. The aim of this study was to analyze the virome of one of the most successful invader in European freshwaters, the signal crayfish (Pacifastacus leniusculus), and shed light on the potential differences in viral sequence composition along its invasion range in the Korana River, Croatia. 120 hepatopancreas tissues were dissected from signal crayfish sampled along its invasion range at four sites: upstream invasion front and core and downstream invasion front and core. Total RNA was isolated, depleted of ribosomal RNA and used for high-throughput sequencing. Subsequent bioinformatics analysis based on protein similarity search of de novo assembled contigs identified novel RNA viruses belonging to reo-like, hepe-like, toti-like and picorna-like viral clades. Sequences of novel signal crayfish associated viruses had relatively low levels of similarity to known viral sequences, however they were phylogenetically related to viruses previously associated with crustacean hosts. The patterns of viral reads abundance and calculated nucleotide diversities of the detected viral sequences varied between the sampling locations along the invasion range, which could indicate the possible influence of different factors and processes on signal crayfish virome composition: e.g., the differences in signal crayfish population density and transfer of viruses from the native co-occurring and phylogenetically related crayfish species. The study reveals a high, previously undiscovered diversity of divergent RNA viruses associated with signal crayfish and set foundations for understanding the potential risk of virus transmissions as a result of this invader's dispersal.

P22

Rapid Pathogen Identification Using Metagenomics Sequencing

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The recent emergence of novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its sub-variants has emphasized the need for rapid non-targeted high-throughput sequencing methods enabling prompt identification of pathogen in the different types of infectious samples. Here we present a wet- and dry- lab protocol, that enables simultaneous DNA and RNA high-throughput sequencing of molecules from highly variable samples that are a mixture of human and microbial origin. Briefly, after total nucleic acid extraction from infectious sample, RNA is converted into cDNA, followed by dsDNA synthesis. The mixture of native DNA and synthetized dsDNA is processed to prepare standard NGS library and sequenced with high-throughput sequencer (Illumina, USA). The generated sequencing data is cleaned of human reads in silico, and the remaining high-quality reads are processed in the downstream analysis, to be aligned to reference microbiome genomes, and quantified. The presented results demonstrate RNA and DNA microbiome species identification and quantification in a single-step sequencing protocol, with sufficient efficiency and accuracy for use in a diagnostic setup.

P23

Comparison Of Milk Kefir Microbiota And Water Kefir Microbiota

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Traditionally, kefir is a fermented beverage, produced by the fermentation with kefir grains that display a symbiotic association of bacteria and yeast, which are enclosed in polysaccharides. Milk kefir and water kefir grains have significant differences in structure, microbial content as well as impact on the fermented beverage. The microbial constitution is also significantly dependent on the origin and local cultivation conditions of the grains. The most common milk kefir grains are Tibetan kefir and Caucasian kefir grains. The microbiota of milk kefir grains mainly contains three groups: lactic acid bacteria, yeasts, and acetic acid bacteria. The most common lactic acid bacteria found in kefir grains include Lactobacillus kefiranofaciens, Lentilactobacillus kefiri, Lentilactobacillus parakefiri, Lactocaseibacillus paracasei, Lactobacillus delbrueckii, Lactobacillus parabuchneri, Lactobacillus acidophilus, Lactiplantibacillus plantarum, Leuconostoc mesenteroides, Lactococcus lactis, Streptococcus thermophilus, Enterococcus durans and others. The main yeast representatives are Kluyveromyces lactis, Kluyveromyces marxianus, Candida kefyr, Pichia ferentans and Saccharomyces cerevisiae. Acetic acid bacteria found in milk kefir are Acetobacter lovaniensis, Acetobacter fabarum and Acetobacter pasterianus. Water kefir grains, also known as sugar kefir or tibicos, are gelatinous grains that contain a synbiotic mixture of bacteria and yeasts embedded in a polysaccharide matrix. Water kefir grains contain more acetic acid species, and more Saccharomyces species than milk kefir grains and less lactococci and Candida species than milk kefir grains. Milk kefir microbiota contain primarily non-Saccharomyces yeasts. The exopolysaccharide in water kefir grains is primarily composed of alpha-glucans, whilst the exopolysaccharide in milk kefir grains is mainly composed of kefiran. Differences in microbial community of these two fermented products shape sensory and technological properties of the product as well as physiological influences on the consumer.

P24

Shear-Induced Hydrodynamic Cavitation For Increasing Biogas Yield In Anaerobic Digestion Process

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Large quantities of wastewater sludge (WWS) that are produced during wastewater treatment need to be stabilized for safe use and disposal. The most common method used for sludge stabilization is anaerobic digestion (AD). Here, microorganisms break down organic matter in the absence of oxygen while also producing biogas, mainly CO2 and methane. Biogas production is driven by complex microbial communities of bacteria, archaea, protozoa, and fungi and can be divided into four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. As hydrolysis is the known limiting step in the AD process substrate pre/treatment various attempts were made to alleviate its influence. Hydrodynamic cavitation was identified as one of the few highly effective tools where cell walls of microorganisms and organic materials are damaged and broken down by mechanical, thermal, and chemical effects of exploding cavitation bubbles, i.e., high local temperatures and pressures with simultaneous OH* production; biofilm matrices, exopolysaccharides, intracellular matter, DNA, are partly released into the bulk liquid, which increases the soluble components (sTOC and sCOD) of substrates amenable for hydrolysis. The actual physical, chemical and interactive effects of shear-induced hydrodynamic cavitation on the mechanisms of AD process were studied to understand the mechanisms in relation to biomethane production. Different cavitation regimes, achieved by altering rotor-stator geometry on a novel Rotary Generator of Hydrodynamic Cavitation (RGHC), were optimized by means of Computer Fluid Dynamics (CFD), high-speed imaging, OH* production, temperature, pressure, and volume flow rate measurements. Effects of cavitation on sludge matrix, its integrity, cell wall damage, intracellular secretions, extracellular polymeric substances (EPS), dewaterability and its overall effect on AD process are currently investigated and evaluated by analysis of physicochemical parameters, characterization of rheological properties, biomethane potential tests, microbiome (distribution of microbial groups, functional genes, antibiotic resistance, virulence genes) and spectroscopic analysis of water-soluble substances (UV-VIS, ExEm).

P25

Microbiota Of Fermented Food Of Animal Origin

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Spontaneously fermented food of animal origin is one of the most complex and dynamic food ecosystems. It may harbor diverse taxa of beneficial, spoilage-associated, and even harmful microorganisms. As for any other complex ecosystem, the characterization of microbial diversity in spontaneously fermented food is still a big challenge. The number of microorganisms in such an environment can exceed 109 CFU/g and the biodiversity still remains largely unknown. Furthermore, the community composition changes rapidly during fermentation and ripening, making the investigation of the microbial diversity of fermented products even more difficult. In our study, we applied a molecular 16S rRNA gene barcoding approach to identify core microbiota and to follow the changes in the community structure during the fermentation and ripening of raw milk cheeses, and dry, wild boar meat sausages. Overall a surprisingly high diversity was found in all analysed food samples. The microbial composition of the milk determined mostly the bacterial community composition in cheese, and overall up to 213 OTUs could be assigned during the ripening of three different types of artisan raw milk cheeses. 20 of the major OTUs were present in all cheese samples and are mostly associated with lactic acid bacteria (LAB), mainly Lactococcus, and Enterococcus species. Also, a large number of non-LAB genera was identified based on phylogenetic alignments, mainly Enterobacteriaceae and Staphylococcacae. In wild boar meat sausages, a total of 83.54% reads was denoted as core microbiota with a remarkable level of Lactobacillus sakei and Lactobacillus curvatus, accounting for 20.55% in initial and 70.48% in ready- to-eat products, and spoilage-associated bacteria including Stenotrophomonas, Bacillus, Pseudomonas, Carnobacterium and Brochothrix, with an average abundance 44.15% at the beginning and 13.98% at the end of ripening.

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Pipelines For Standardized Large Microbiome Data Analysis Prepared For HPC Computing – From 16S To Mags

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Microbiology has shifted towards analyses of large data sets containing thousands of samples utilizing high performance computing (HPC). Large datasets enable extensive biomarker search and mechanistic insight through the adoption of advanced statistics and machine learning approaches. In order to alleviate the observed bottleneck and resolve reproducibility issues three HPC compatible pipelines were prepared for the analysis of microbial datasets that include the most cited and already used programmes. GUMPP (General Unified Microbiome Profiling Pipeline [doi:10.3390/metabo11060336] takes amplicon sequencing data through Mothur-based taxonomic analyses at four different levels (genus / species/97% operational taxonomic units / amplicon sequence variants), calculates a variety of alpha-diversity indices and inputs BIOM file into Picrust2 for the prediction of microbiome functional genes, enzymatic reactions and metabolic pathways. HPCmetaBakery utilizes bioBakery tools for large scale analyses of metagenomics sequencing data and produces species level taxonomy, microbiome functional genes, enzymatic reactions, metabolic pathways and human gut predicted metabolites. MAGO (Metagenome Assembled Genomes Orchestra) [doi:10.1093/molbev/msz237] further extends metagenomics data analysis towards quality control, sequence assembly, binning and evolutionary analysis of metagenome assembled genomes. To facilitate HPC adoption, maximise the use of resources and reproducibility all pipelines are prepared as Singularity containers with config file that runs run the entire pipeline, enables customisation and can be shared with other researchers. The resulting matrices are compatible with modern statistical approaches and machine learning methods to build classification models, search for biomarkers in standardised way on large datasets produced globally in order to improve our understanding of microbiome influence on human health and environment.

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HPC In Biomed: From Bacterial Taxonomy To Functional Gene Microbiology And Machine Learning

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To facilitate the study of microbial populations and their functional potential in such research environment, we developed the General Unified Microbiome Profiling Pipeline (GUMPP). GUMPP is a workflow designed for integrated analysis of bacterial 16S rRNA to taxonomy by building on the Human Microbiome Project procedures for amplicon sequencing data analysis in the Mothur program, and extending these to the predicted functional genes, enzymatic reactions and metabolic pathways using Picrust2. Outputs for metagenome predictions using the Piphillin web server are also enabled. The entire workflow is available as a Singularity image including config files for portability from to High Performance Computing (HPC) clusters, for teaching and research purposes. The Singularity image consists of the Mothur and Picrust2 programs, the required Mothur scripts, two Silva taxonomy databases, and a skeleton framework consisting of more than 9000 lines of Python code. GUMPP is the first workflow to introduce HPC level traceability of the analytical parameters, that can be shared alongside with the data for increased reproducibility to support data integration (metagenomics, metaproteomics, metabolomics) and the identification of important biochemical pathways for specific medical pathologies. GUMPP also alleviates the inefficient utilization of HPC resources by the current state-of-the-art programs by adopting orchestrated execution of software pieces using ExeFlow within which each execution entity has performance attributes that hint about its CPU and disk utilization, based on which a scheduler selects pieces for parallel execution and strives to utilize available resources thoroughly but without saturating the system.

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Metagenomics Data Analysis Software: Trouble In HPC Paradise

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As soon as the working of a software in question suits some specific purpose, its development ceases /slows down due to preoccupation of developers with other activities. To address the problem a framework for production and analysis of Metagenome Assembled Genomes was designed. Disk consumption was vastly larger than needed since software blindly produced many unnecessary output files, many pieces of software were parallelized inefficiently generating significant computational bottlenecks. This work was extended to KneadData preprocessor (BioBakery workow, https://github.com/biobakery/biobakery) consisting of Trimmomatic quality control, Bowtie2 elimination of contaminants and a single-threaded TRF tandem repeats removal. Results of several Bowtie2 runs were combined with an inefficient single-threaded Python script. As improvement, TRF was parallelized, whereas Bowtie2 results were combined by a C++ program. Consequently, our inhouse ExeFlow was designed for orchestrated execution of various unrelated software pieces. Each execution entity has performance attributes that project its CPU and disk utilization, a scheduler that selects pieces for parallel execution and strives to utilize available resources without saturating the system, all accompanied by controlling script, which determines optimal commandline parameters (e.g. to generate only the needed resulting files) and deletes unneeded resulting files immediately upon completion of the associated step. One metagenomic solution is currently under development on the basis of the ExeFlow workflow, MetaBakery reimplementation of BioBakery workflow, while GUMPP General Unified Microbiome Profiling Pipeline is complete.

P29

Sterilization Of Delicate Cellulose Materials For Application Of Radio Frequency Oxygen Plasma

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The conventional sterilization methods such as autoclave and UV irradiation can change the surface and bulk properties of delicate organic polymers. By applying those methods on medical textiles inactivates the bacteria, but the residue of highly dangerous bacteria can still persist on the surfaces of the polymer. This study shows the advantage of using plasma afterglow as a sterilization method of cellulose materials used for medical devices. Cellulose material was treated in highly reactive oxygen plasma under different plasma parameters. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) was used to determine the effect of gaseous plasma reaction on cellulose material and their effect on the surface. Inductively coupled radio frequency (RF) oxygen plasma afterglow was supplying the flux of neutral oxygen atoms. The afterword treated cellulose material was exposed to bacteria Escherichia coli (E.coli), strain ATCC25922, which was grown in a solution of Luria-Bertani (LB) medium. The SEM images revealed huge differences in morphology of cellulose treated by oxygen plasma using different plasma parameters as well as differences in bacterial attachments. While oxygen ions in plasma glow tend to drill holes into the bacterial cell wall and here, we observe it also destroys into cellulose material, the atoms in plasma afterglow cause more even disruption of bacterial cell wall leaving cellulose material intact. The results of plasma parameters used in gentle material were explained by kinetic properties of plasma, potential mechanisms and effects of changes.