Microbiology day 2023

Preliminary program:
8:30-9:00 Morning coffee / breakfast
9:00-9:10 Welcome words
9:10-9:50 Keynote 1: Environmental microbiology, Alexander Tveit UiT, The Arctic University of Norway
9:50-10:10 Agate Auzane
10:10-10:30 Muhammad Suleman Qasim
10:30-10:50 Melina Markkanen
10:50-11:10 Sirja Viitamäki
11:10-12:10 *Lunch*
12:10-12:50 Keynote 2: Molecular microbiology, Juha Saarikangas, University of Helsinki
12:50-13:10 Victor Manuel Gonzalez Ramos
13:10-13:30 Ashwini Kedari
13:30-13:50 Xiaodan Ouyang
13:50:14:10 Maija Suvanto
14:10-14:35 *Afternoon coffee*
14:35-15:15 Keynote 3: Human / clinical microbiology, Marja Roslund LUKE (Natural Resources Institute Finland)
15:15-15:30 Poster pitches
15:30-15:50 Vy Anh Huynh
15:50-16:10 Roosa Jokela
16:10-16:30 Ravi Kant
16:30-16:50 Vinaya Venkat
16:50-17:00 Break
17:00-19:00 Coctails and posters

Event date: Thursday 11th May 2023 at Info center, Viikki campus.

The best talk and poster will be awarded a prize of 1 000€ travel grant.

The Microbiology Day 2023 organizers:
Jenni Hultman PI, Senior Scientist, LUKE.
Muhammad Suleman Qasim Doctoral researcher MBDP programme.
Katariina Lahti-Leikas Doctoral researcher MBDP programme.

MBDP doctoral programme.

ISME MBDP Doctoral Programme in Microbiology and Biotechnology UNIVERSITY OF HELSINKI

Microbiology day 2023
11 May 2023

Abstracts for talks

Session 1: Environmental microbiology.

Presenter: Agate Auzane

Title: Understanding mechanisms behind interactions of yeast-like fungi in the order Taphrinales

Session: 1. Environmental microbiology

Abstract:

Yeasts and yeast-like fungi are understudied but important plant-associated microbes that can act as pathogens, commensal residents, or beneficial symbionts. Members of the Ascomycete order Taphrinales include Taphrina and Protomyces species. These phytopathogenic yeast-like fungi are dimorphic and often cause tumours on host plants. Members of these genera are known to alter plant phyllosphere microbiome structure. To utilize model system genetics, we isolated yeasts and yeast-like fungi associated with wild individuals of the genetic model plant Arabidopsis thaliana (Arabidopsis). These included a novel strain M11 of Taphrina potentillae and the new species, Protomyces arabidopsidicola, which was found to be among the most commonly isolated fungi from Arabidopsis leaves. We have sequenced the genomes of three Taphrina species and seven Protomyces species. Here we focus on genome analysis and host plant interactions. P. arabidopsidicola was not pathogenic on Arabidopsis but could persist on the leaf surface and activated immune signalling. Infection of Arabidopsis with M11 resulted in disease symptoms including subtle leaf curling, reminiscent of leaf deformation symptoms caused by other Taphrina species. Genomic analysis revealed genomic features associated with host interaction, including putative effectors and potential MAMPs recognized by the plant innate immune system. We are utilizing these experimental systems for genetic screens to identify loci involved in the immunity against and perception of plant-associated dimorphic fungi and yeasts. In conclusion, we have established a model system for the study of the interaction of yeast-like fungi with Arabidopsis. Use of this system will help define the genetics of plant-yeast interactions.

Presenter: Melina Markkanen

Title: Using metagenomic long-read sequencing combined with DNA methylation information

Session: 1. Environmental microbiology

Abstract:

Antibiotic resistance poses a tremendous threat to the health care system globally. The ability to resist antibiotics is achieved by genomic changes in the bacterial DNA e.g., by the acquisition of genetic elements carrying antibiotic-resistance genes (ARGs). A wide array of ARGs have been detected in wastewater even after treatment. However, knowledge about the genetic context and host bacteria of these genes is often lacking but required to be able to assess the risks caused by the ARGs found in wastewater and potentially wastewater-receiving environments. Linking ARGs to their genetic context or host bacteria in metagenomic data is still methodologically challenging. DNA methylation motifs and their methylation frequency vary among different bacteria. Unique methylation profiles can be used as signals for distinguishing DNA fragments based on their origin. In this study, we used PacBio metagenomic long-read sequencing combined with DNA methylation information to investigate ARGs and their genetic contexts and possible hosts throughout the wastewater treatment process. Among other ARGs, we focused on sul4 which is a recently discovered sulfonamide resistance gene with unknown host bacteria and genetic context. Our results based

on long-read assembled contigs, their methylation profiles and taxonomical identification suggest that the sul4 gene is carried by various taxa and it is in all cases closely linked to an integrase element. The research on bacterial methylation has mainly focused on isolates. However, as we show here, methylome analysis of metagenomic DNA shows potential for higher resolution genome-resolved metagenomics and host prediction of antibiotic resistance genes.

Presenter: Muhammad Suleman

Title: tRNA modifications under diverse environmental conditions in Shewanella bacteria

Session: 1. Environmental microbiology

Abstract:

The genus Shewanella includes bacteria inhabiting diverse marine environments. They can be psychrotrophic or mesophilic and are known to tolerate the freezing temperatures of the Arctic. The aim of my project is to evaluate how changes to the global post-transcriptional nucleoside modification (PTMs) pattern on tRNA molecules in the cold-active Shewanella glacialimarina TZT-4T bacterium affect stress responses to divergent environmental conditions, i.e., temperature, pH, and salinity. For this purpose, I cultivated bacterial cells at different temperatures (0-25°C), salinity (0-6% w/v NaCl), and pH (5.5-10) conditions followed by acidic phenol extraction and ion-exchange chromatography of bulk tRNA. To investigate translational adaptation strategies to environmental changes, I performed a quantitative analysis of tRNA modification dynamics upon stress. My preliminary results show a clear temperature dependence for some translation-modulating anticodon modifications, such as inosine (I) and queuosine (Q) that peak at 5°C and 25°C, respectively. Moreover, I also observe a significant increase in 2'-O-methylcytidine (Cm) and 2'-O-methylguanosine (Gm) at high NaCl concentrations, possibly suggesting a subtle stabilizing effect on translation during stress. This study will also utilise Shewanella and Escherichia coli-based cell-free translation systems, which provide a real-time, robust platform for assessing translation kinetics. The development of this system will establish an open environment for monitoring translation and enables the incorporation of differentially primed tRNA with stress related PTMs for their role as translational modulators.

Presenter: Sirja Viitamäki

Title: Wintertime microbial activity in Finnish subarctic tundra soils

Session: 1. Environmental microbiology

Abstract:

Due to climate change, increased microbial activity in high-latitude soils might lead to higher greenhouse gas emissions. However, microbial greenhouse gas production and consumption mechanisms in tundra soils are poorly studied, especially during winter. We aimed to provide new information on wintertime microbial activity concerning greenhouse gas production and consumption in subarctic tundra soils. To investigate the diversity and functions of bacterial and archaeal communities, we analyzed 17 soil metatranscriptomes collected in early April from Kilpisjärvi, northern Finland. Fluxes of greenhouse gases nitrous oxide (N2O), methane (CH4), and carbon dioxide (CO2) were measured. Also, pH, soil organic matter, and soil water content were measured. Actinobacteria, Acidobacteria, and Alphaproteobacteria were the most active phyla, dominated by aerobic acidophilic genera that play a role in the degradation of plant organic matter. The most abundantly transcribed genes were involved in genetic information processing and energy and carbohydrate metabolism. CO2 fluxes were positive and N2O fluxes varied from negative to positive, whereas CH4 fluxes were mostly negative. Genes encoding methane monooxygenase (pMMO) were transcribed in

some soils. These results indicate microbial activity and microbial decomposition of plant litter in tundra soils in early April. Along with climate warming, increased decomposition may affect the rate of carbon turnover in tundra soils. Transcription of methane oxidation genes together with methane uptake in soils show that these tundra soils are a possible sink of atmospheric methane in early spring.

Session 2. Molecular microbiology.

Presenter: Victor Manuel Gonzalez Ramos

Title: Genetic manipulation of white-rot fungus Dichomitus squalens using CRISPR/Cas9

Session: 2. Molecular microbiology

Abstract:

The basidiomycete white-rot fungus Dichomitus squalens is an efficient wood-degrading species which produces a highly adjusted enzymatic response to various types of plant biomass. Detailed bioinformatic analyses of recent (post-)genomic studies in this fungus have revealed candidate regulators of the enzymes involved in the lignocellulose breakdown. However, functional characterization of genes encoding the lignocellulose-degrading enzymes and their regulators require efficient and reliable genetic manipulation tools. Currently, the genetic manipulation of basidiomycete white-rot fungi is still hampered by extremely low frequencies of homology-directed recombination (HDR) and limited number of available selection markers. Different CRISPR/Cas9-based genome editing methodologies have recently been developed for genetic manipulation of a few basidiomycete fungi, such as for the species from the genera Ganoderma, Pleurotus, Ustilago and Schizophyllum. The current strategies vary in Cas9 expression system, single guide RNA (sgRNA) synthesis, and HDR template type. Additionally, the disruption of the non-homologous end-joining machinery is used for increased efficiency of HDR. The established approaches show promise on deletion, insertion, substitution, and disruption of single or multiplexed genes for their in vivo functional characterization. In D. squalens, we have established a CRISPR/Cas9-based genome editing approach that utilizes Cas9-sgRNA ribonucleoproteins with single-stranded oligodeoxynucleotides as donors for HDR. Through the targeted introduction of STOP codons, we have successfully disrupted genes encoding candidate transcriptional regulators and lignocellulose degrading enzymes. We will showcase the development of the targeted CRISPR/Cas9 approach in D. squalens and its application in the analysis of gene functions and discuss the challenges in genetic manipulation of basidiomycete (wood-degrading) fungi.

Presenter: Ashwini Kedari

Title: Insight into rabies virus neutralization revealed by engineered antibody scaffold

Session: 2. Molecular microbiology

Abstract:

Rabies virus (RABV) is a lethal neurotropic virus which causes approximately 59000 deaths annually. The trimeric glycoprotein (G) spikes of RABV are the only surface-exposed proteins on the virion and play a significant role in mediating receptor recognition and host cell entry. RABV-G displays several epitopes which are targeted by neutralizing antibodies, including monoclonal antibodies (mAb) that have been proposed as cost-effective alternatives to traditional polyclonal rabies immunoglobulin treatment. In this study, one such neutralizing antibody was engineered into a diabody to eliminate the flexibility possessed by a full-length mAb and facilitate rapid crystallization. Here, we report the crystal structure of the RABV-G subdomain in complex with the diabody at 3.15 Å resolution. The structure reveals a key part of the antibody epitope which targets a 6-residue hydrophobic peptide on the RABV-G subdomain. The antibody interacts with the antigen

primarily via hydrophobic interactions mediated by complementarily determining regions heavy chain 2 and 3, and light chain 3. Our results provide a structural description for rabies inhibition by one of the most cross-neutralizing antibodies against the diverse rabies strains.

Presenter: Xiaodan Ouyang

Title: Direct pathway cloning and expression of the radiosumin biosynthetic gene cluster

Session: 2. Molecular microbiology

Abstract:

Radiosumins are a structurally diverse family of low molecular weight natural products that are produced by cyanobacteria and exhibit potent serine protease inhibition. Members of this family are dipeptides characterized by the presence of two similar non-proteinogenic amino acids. Here we used a comparative bioinformatic analysis to identify radiosumin biosynthetic gene clusters from the genomes of 13 filamentous cyanobacteria. We used direct pathway cloning to capture and express the entire 16.8-kb radiosumin biosynthetic gene cluster from Dolichospermum planctonicum UHCC 0167 in Escherichia coli. Bioinformatic analysis demonstrates that radiosumins represent a new group of chorismate-derived non-aromatic secondary metabolites. High-resolution liquid chromatography-mass spectrometry, nuclear magnetic resonance spectroscopy and chemical degradation analysis revealed that cyanobacteria produce a cocktail of novel radiosumins. We report the chemical structure of radiosumin D, an N-methyl dipeptide, containing a special Aayp (2-amino-3-(4-amino-2-cyclohexen-1ylidene) propionic acid) with R configuration that differs from radiosumin A-C, an N-Me derivative of Aayp (Amyp) and two acetyl groups. Radiosumin C inhibits all three human trypsin isoforms at micromolar concentrations with preference (from 1.7 μM to > 7.2 μM). These results provide a biosynthetic logic to explore the genetic and chemical diversity of the radiosumin family and suggest that these natural products may be a source of drug leads for selective human serine proteases inhibitors.

Presenter: Maija Suvanto

Title: Characterization of two orbiviruses isolated from Finnish Ochlerotatus communities

Session: 2. Molecular microbiology

Abstract:

Orbiviruses (family Sedoreoviridae) are dsRNA viruses with a 10-segment genome, that infect a wide range of vertebrates, including humans. These viruses have worldwide distribution and are transmitted via haemophilic arthropods, e.g., ticks, and mosquitoes.

In our virus isolation trials following our mosquito virome study of Finnish mosquitoes, we detected two orbiviruses. Based on preliminary sequence analysis, one of them was found to be a novel strain of orbivirus and the second orbivirus was determined to be a strain of Mobuck virus. The aim of this study was to characterize the above orbivirus isolates.

Virus isolations were conducted by inoculating mosquito-derived C6/36 and mammalian Vero E6 cells with homogenised specimens of Ochlerotatus communis through two passages. No cytopathic effect (CPE) was observed in Vero E6 cells, but CPE was observed in C6/36 cells during the second passage.

Genome characterizations are ongoing, but in the preliminary phylogenetic analysis, the novel orbivirus clustered with CHeRI orbiviruses and Mobuck virus strain clustered with known Mobuck virus isolates. The

most closely related CHeRI orbiviruses and Mobuck viruses were isolated from dead white-tailed deer in the USA, but vectors are not yet established.

The Finnish Mobuck virus strain widens the known distribution of Mobuck virus to outwit the USA. Yet, there is no evidence that either isolated orbiviruses infect mammalian cells in vitro. However, the genetic relatedness to viruses with mammalian host associations makes them potential pathogens of interest. Further studies are needed to determine the host range and possible vertebrate disease associations of both viruses.

Session 3. Human / clinical microbiology.

Presenter: Vy Anh Huynh

Title: Desulfovibrio bacteria and Parkinson's disease

Session: 3. Human / clinical microbiology

Abstract:

Parkinson's disease (PD) is a common and complex neurodegenerative disorder that affects the movement. The root causes of the disease have remained unknown despite more than 200 years of intensive studies. It has been suggested that genetics, environmental factors and lifestyle play some role in the disease pathogenesis. Accumulation of the neuronal protein alpha-synuclein (alpha-syn) is the key pathological feature of PD. The aggregates have been found not only in the central nervous system, but also in other parts of the human body including gastrointestinal tract. Alpha-syn aggregation, thus, has been suggested to be induced in the gut cells by intestinal pathogens. Furthermore, exposure to various microbial components and products (namely curli-producing Escherichia coli) has been shown to trigger alpha-syn aggregation and PD-like symptoms in animal models. Recently, we showed that Desulfovibrio bacteria are associated with PD. However, it was not clear how these bacteria contribute to the disease, particularly alpha-syn pathology. In this study, by employing a Caenorhabditis elegans model that overexpresses human alpha-syn, Desulfovibrio bacteria isolated from the feces of PD patients have been demonstrated to increase alpha-syn aggregation in both volume and abundance, statistically more than curli-producing E. coli. Additionally, patient Desulfovibrio strains were stronger than those isolated from healthy individuals in inducing alpha-syn aggregation and toxicity. Our results suggest that Desulfovibrio bacteria, especially patient strains, contribute to PD pathogenesis by inducing alpha-syn aggregation.

Presenter: Roosa Jokela

Title: Sources of gut microbiota variation in a longitudinal Finnish infant cohort

Session: 3. Human / clinical microbiology

Abstract:

Although the infant gut microbiota has been extensively studied, comprehensive assessment on the microbiota covariates including technical variables has not been performed in large infant cohorts. We studied the effect of 109 variables on the 16S rRNA gene amplicon-based gut microbiota profiles of infants sampled longitudinally over the first two years of life in the Finnish HELMi birth cohort. Spot faecal samples from both parents were included for intra-family analyses, totaling to 7660 samples from 985 families that were evaluated for beta diversity patterns using permutational multivariate analysis on Bray-Curtis distances, and differential abundance testing and alpha-diversity for variables of interest. In time point-specific models, the largest effect sizes, up to 6%, were seen for the DNA extraction batch, delivery mode and related perinatal

exposures, defecation frequency and parity/siblings. Variables describing gastrointestinal function were consistently important during the first two years, reflecting changes in e.g., feeding habits. The effect of parity on infant microbiota was modified by birth mode and exposure to intrapartum antibiotics, exemplifying the tight interlinkage of perinatal factors relevant for infant microbiota research. In total, we were able to characterize up to 19% of the total biological bacterial variation in the infant gut. Our results highlight the need of result interpretation in the context of each cohort's characteristics. Our study provides a comprehensive report of key covariates of infant gut microbiota composition across the two first years of life in a homogenous cohort. The study highlights possible important future research areas and confounding factors to be considered.

Presenter: Ravi Kant Ojha

Title: SARS-CoV-2 cell entry, infection, and host targeting antivirals

Session: 3. Human / clinical microbiology

Abstract:

Both SARS-CoV and SARS-CoV-2 use ACE2 as a receptor for cellular entry and spreading. However, tissue tropism of these two viruses differs. The spike (S) protein of SARS-CoV-2 contains a furin cleavage site that is absent in SARS-CoV. Cellular protein Neuropilin-1 (NRP1) is known to bind furin-cleaved substrates. In our study, we found that NRP1 significantly potentiates SARS-CoV-2 infection. The infection was blocked by monoclonal antibody against NRP1. A mutant virus lacking the furin cleavage site did not depend on NRP1 for infectivity. The S protein of SARS-CoV-2 undergoes proteolytic cleavage by the host cell proteases cathepsin and TMPRSS2 for activation and efficient infection. Apilimod, a PIKfyve inhibitor, blocks the late endosomal viral movement and prevents in vitro infection mediated by cathepsin. Camostat mesylate or nafamostat mesylate inhibit TMPRSS2 activity and prevent infection mediated by the TMPRSS2-dependent pathway. When we combined apilimod with either of the two drugs, the effectiveness increased by 5-10fold to prevent SARS-CoV-2 infection in vitro. There are many ongoing efforts to discover effective antiviral drugs that could help control the severity of the disease. The above-mentioned drugs serve as promising candidates to suggest a treatment strategy based on the combined use of these virus inhibitors.

Presenter: Vinaya Venkat

Title: Understanding infection dynamics of SARS-CoV-2 Omicron variant

Session: 3. Human / clinical microbiology

Abstract:

With the inception of the COVID-19 pandemic, the causative agent SARS-CoV-2 has been mutating in its infection host – humans. The variants have their own characteristics of infection due to divergence from the wild type. The current variant of concern – Omicron, highly diverged from the wild type and other variants, is said to be more transmissible in humans. To understand why this is the case, a study of SARS-CoV-2 Omicron variant infection using mink as an animal model was conducted to determine the transmission of the virus and pathogenesis in infected tissues. The experiment lasted for a short period, with hints at gender bias in infection, which is being verified by transcriptomics, to understand gene regulation during an infection – affecting disease susceptibility and immune response. The experiment will also prove useful to study virus evolution in mink, if any. Further, a longer infection experiment is planned to study the complete course of infection and monitor the production of antibodies against the virus as part of the immune response.

Abstracts for posters

Session 1: Environmental microbiology

Presenter: Silvia Cera

Title: Tailored oat fermentation processes improve texture, quality and flavour of 100%

Session: 1. Environmental microbiology

Abstract:

Background Lactic acid bacteria (LAB) and yeasts have traditionally been the main actors in grain fermentation processes. Sourdough fermentation promotes positive changes in cereals, such as the enhancement of flavour and texture and the improvement of nutritional properties. Oat is an ideal grain due to its valuable nutritional profile and β-glucan cholesterol lowering properties; however, the lack of gluten makes the use of oat challenging. Objectives To investigate how tailored fermentation processes induced by selected starters can improve texture, quality and flavour of 100% oat sourdoughs and bread. Methods The evaluation of LAB fermentation performance in wholegrain oat flour led to the selection of Weissella confusa VIII40 and Leuconostoc citreum 5B8, as dextran producers, while Levilactobacillus brevis IC9, Lactiplantibacillus plantarum 1MR20 and Saccharomyces cerevisiae LNE10 were selected as consortium to modulate sourdough flavour. Dextran was quantified via HPAEC-PAD. Baking tests, bread quality measurements (texture profile analysis) and sensory analysis were conducted to assess the different sourdoughs effects. Results All sourdoughs reached pH ca. 4 and moderate acidity. Dextran was approximately 2-4% of dry weight depending on the strain used. Sourdough breads containing dextran were softer and had lower staling rate than control. Sensory evaluations of sourdoughs fermented with different starters associations and related breads showed significant differences depending on the combination used. Tailoring the fermentation processes of oat permitted us to achieve all the objectives established.

Presenter: Maria Christodoulou

Title: New taxa of terrestrial rock-inhabiting cyanobacteria from Finland

Session: 1. Environmental microbiology

Abstract:

Cyanobacteria represent a group of photosynthetic bacteria found in almost all habitats on Earth including those, which are considered hostile to life from our anthropocentric point of view and are known as ‘extreme’ environments. Caves, cave-like environments, and rocks represent examples of such ecosystems. Cyanobacteria thriving in aquatic or soil habitats have been extensively studied. However, the number of studies dealing with terrestrial rock-inhabiting cyanobacteria is limited. The aim of this work is to investigate the diversity of epilithic filamentous cyanobacteria from non-studied terrestrial habitats in Finland. Environmental samples were collected using sterile tools from different locations in Southern Finland including the historically and culturally important Suomenlinna Fortress (UNESCO). Strains were isolated and studied using a polyphasic approach, which included morphological (light microscopy), molecular (16S rRNA, 16S-23S ITS) and ecological data. Thirty strains of filamentous cyanobacteria were isolated for the first time from wet and dry rocks as well as other sciophilic habitats in Finland. Nine of these strains belong to the order Nostocales and were morphologically close to Nostoc, Calothrix and Roholtiella whereas the remaining 21 strains resembled Leptolyngbya, Pseudanabaena Phormidesmis and Phormidium. Phylogenetic analysis based on 16S rRNA and 16S−23S ITS secondary folding structures, indicate the existense of at least 3 new

genetic lineages and at least 12 new species. These findings contribute to expanding cyanobacterial diversity and highlight the importance of studying terrestrial rock-inhabiting cyanobacteria.

Presenter: Laura Häkkinen

Title: Land use effects on fungal community structure in soil vertical profile

Session: 1. Environmental microbiology

Abstract:

Regenerative farming practices are known to increase soil biodiversity and soil carbon content by soil organic matter (SOM) accumulation. Impacts of different farming practices on soil microbial community and carbon have mostly been studied in topsoil. Comprehensive view of the farming practices requires that the effects on deeper layers of soil vertical profile are also monitored. Here, four soil types, organic, conventional, meadow, and forest, were analyzed with ITS amplicon sequencing to 80 cm deep. Soil properties and root biomass were measured to investigate how land use and environmental factors drive changes in fungal community structure in the soil vertical profile. We found that fungal community differences were best explained by depth and soil type, and that vegetation, pH, phosphorus, carbon and root biomass contributed to a small additional effect on fungal community differences. Soil type effect on fungal community differences was detected in all soil layers indicating land use effects on fungal community across soil profile down to 80 cm. The highest fungal diversities were found in the organic and conventional topsoil layers while meadow was the most diverse in the 30-40 cm soil layer. There were no differences in fungal diversity between soil types in the deepest soil layer. Arbuscular mycorrhiza forming Glomeromycota relative abundances increased towards deeper layers of soil, were lowest in the forest soils, and highest in meadow and organic deep soil layers indicating that the less intense land use increased the presence of beneficial arbuscular mycorrhizal fungi.

Presenter: Matilda Kattilakoski

Title: Long-term effects of wood-ash fertilization on peatland forest soil microbiome

Session: 1. Environmental microbiology

Abstract:

Wood-ash contains essential mineral nutrients for tree growth but no nitrogen. It is commonly used fertilizer in drained peatland forests as these forests do not lack nitrogen. In the efforts of mitigating climate change, the use of renewable energy sources has increased leading to increased amounts of byproducts, such as ash from biofuel combustion. Increasing tree growth is a central tool to capture carbon in the land use sector. Large increase in the use of ash fertilizers in drained peatland forests to increase carbon capture is proposed in Finland. As soil microbial communities are responsible for the nutrient and carbon cycles in soil it is crucial to know how they react to ash fertilization. This study investigates the effects of wood-ash fertilization on soil microbiome composition, structure, and functional potential in drained peatland forests. Soil samples were collected from six different locations in Finland in the fall of 2022. Each site had a wood-ash fertilized site and a non-fertilized control site next to it. Six samples were collected from each fertilized and each control site adding up to a total of 144 samples. DNA was extracted from these samples and shotgun sequenced using Illumina sequencing. This data will be analyzed using metagenomic approach to investigate the taxonomic community structure and composition as well as functional potential of the sampled communities. We expect to gain novel knowledge about the long-term effects of wood-ash fertilization on soil microbial community structure, composition, and functional potential in drained boreal peatland forests.

Presenter: Eero Kiviniemi

Title: BIOACTIVE NATURAL PRODUCTS FROM LABORATORY CULTIVATIONS OF WOOD DECOMPOSING FUNGI

Session: 1. Environmental microbiology

Abstract:

Wood decaying fungi of Basidiomycota are among the most important organisms in the forest ecosystems. The fungi modify and decompose deadwood and its constituents (cellulose, hemicelluloses and lignin), and recycle the deadwood carbon and nutrients for themselves and other organisms. Wood decaying fungi produce a wide variety of bioactive secondary metabolites. These organic compounds represent terpenoids, polyketides, alkaloids, peptides, and polyphenols. These metabolites may be utilized by the fungi e.g. as redox-active agents in wood decomposition reactions, as antimicrobial or antifungal compounds against other microbes when competing for the scarce resources available in deadwood. Despite their importance for the forest ecosystems, and their vast repertoire of bioactive compounds, the metabolic capabilities of wood-decaying fungi have been largely understudied, with many of the studies focusing on compounds found in their fruiting bodies. Environmental stimuli and changes such as atmospheric, temperature and pH fluctuations, microbial interactions, scarcity of nutrients, and light have been recognized as key factors affecting the production of secondary metabolites, especially in filamentous fungi of the phyla Ascomycota [1]. For Basidiomycota fungi, systematic studies on regulation of secondary metabolism have been performed for only a few species. Our research has focused on the genomics and metabolism of wood-decay fungi which are commonly found in Finland and present different degradation mechanisms and lifestyles [2,3]. On this presentation we show our findings on the effect of stress on fungal activities and production of secondary metabolites. Biotechnological potential for sustainable production of fungal bioactive natural products in laboratory scale is also assessed. [1] Keller NP. Nature Reviews Microbiology, 17:167-180, 2019. [2] Mäkinen M, et al. BMC Genomics, 20:430, 2019. [3] Mali T, et al. Fungal Ecology, 61:101199, 2023.

Presenter: Veronika Lyasnikova

Title: Horizontal gene transfer in the presence of metals in a microbial community

Session: 1. Environmental microbiology

Abstract:

The spread of antibiotic resistant bacteria and antibiotic resistance genes in the environment is a global concern for human health. The human use of antibiotics and metal containing fertilizers causes the release of antibiotic and metal resistance genes into agricultural soils and wastewater, which triggers the development of antibiotic and metal resistance in microorganisms. It is believed that the increase of metal concentrations in soils and wastewater might affect the antibiotic resistance of the bacteria populating these environments. To test that, we have planned an evolutionary experiment where an artificial microbial community from different strains is cultivated with a donor strain carrying a conjugative plasmid encoding resistance to several antibiotics. During a series of laboratory evolutionary experiments, the bacterial community will be exposed to sub-inhibitory concentrations of antibiotic solutions and metals ions (copper or nickel). The results of the experiment will provide information on how metals affect the horizontal gene transfer of antibiotic resistance genes and the diversity of the microbial community. My research project will provide more information on the relationship between metal and antibiotic resistance, as well as understanding of the mechanisms and dynamics of antibiotic gene transfer.

Presenter: Renata Majamäki

Title: Baltic Sea ferromanganese concretions as a sustainable source of hi-tech metals

Session: 1. Environmental microbiology

Abstract:

The transition to renewable energy and the acceleration of technology demand vast amounts of hi-tech metals. More than 10% of Finland's sea areas are covered by a layer of ferromanganese concretions, which are centimeter-scale accumulations of iron and manganese oxides. In addition to iron and manganese, concretions contain hi-tech metals, such as cobalt. Due to increasing demand for hi-tech metals, rising interest to start mining concretions from the Baltic Sea is inevitable. The concretions host diverse microbial communities that affect the concretion growth and dissolution rates. It is crucial that the ecosystem is not damaged while concretions are extracted. This project provides information on microbial communities occupying the concretions, their metabolic functions, and hi-tech metal accumulation and release mechanisms in concretions. With the results, we can estimate the recovery rates of Baltic Sea concretions after seabed mining. The ferromanganese concretions were collected from the Baltic Sea during May-June 2022 for laboratory incubation and metal tracer experiments. We assessed the concretion growth with nano-computed tomography and a scanning electron microscope, and measured the production of methane, carbon dioxide, and nitrous oxide. Metal accumulation will be assessed based on the incubation solutions' and concretions' geochemical analysis. Active microbial community members and their functions will be analyzed through metatranscriptomics. The preliminary results provide new information on the growth rates and conditions of concretions. Clear growth in the concretions was detected in the images taken with a nano-CT before and after the 12-week incubations.

Presenter: Meri Salomaa

Title: Persistent dsRNA bacteriophage infection

Session: 1. Environmental microbiology

Abstract:

Persistent infection, in which a pathogen is continuously present in the host organism, is a frequent phenomenon in virus-host systems. Persistent viral infections are expected to play a significant role in the ecology and evolution of their host organisms, but our understanding of these prolonged virus-host interactions is still limited. In our study, we characterized the persistent infection strategy of Pseudomonas phage phi6. Phi6 is a lytic virus that can also establish a carrier cell state in which viruses form within the host cell without causing cell lysis. We found that the persistent infection of phi6 did not affect the growth of the host bacteria. The stability of the carrier cell was shown to be temperature sensitive and was enhanced at elevated temperature both in nutrient rich and poor conditions. In addition, we discovered that the phi6 carrier cell infection gave resistance to the host bacterium against secondary infections of the same phage but not against related phages. Phi6 S-segment was needed for this superinfection exclusion effect. More specifically, S-segment gene knockout assays suggest that the superinfection exclusion is mediated by phi6 gene 8, which codes for the major outer capsid protein.

Presenter: Rashmi Shrestha

Title: Microbiological impacts of plant diversity to soil carbon sequestration

Session: 1. Environmental microbiology

Abstract:

Most agricultural soils globally loose carbon continuously due to conventional cultivation methods. Using diverse cover crops and avoiding winter bare fields may prevent carbon loss leading to improved soil health and climate change mitigation. The fate of soil carbon relies on microbial activity. Soil microbes use most plant-derived carbon and either produce CO2 or incorporate it into their biomass and after death, microbial necromass may contribute to stable carbon. Diverse cover crops may alter microbial community diversity and function by diversifying microhabitats with varying root exudates and litter. They may ensure maintenance of more, different, and beneficial (arbuscular mycorrhizal fungi) microbial populations throughout winter through nourishment. Legumes fix atmospheric nitrogen (N) making it available to plants, whereas grasses take up excess soil inorganic N and help in N retention. One cover crop species cannot provide full set of ecosystem services. Using mixtures of functionally diverse cover crop species may benefit soil microbes and contribute to varied ecosystem services and this remains unexplored. My PhD project aims to investigate the effect of plant diversity on agricultural soil microbial community composition and activity related to carbon cycle. Here, we used an experimental field where barley is undersown with 1, 2, 4 and 8 cover crop species varying in their functional traits (deep and shallow rooted grasses and legumes). We collected soils during 2019-2020 period to determine microbial biomass, respiration, microbial community composition and carbon use efficiency. The results will show if diverse cover-cropping is suitable climate smart practice in boreal climate.

Presenter: Anne Tyvijärvi

Title: Complex formation between fungal necromass and condensed tannins – a missing piece in the soil organic matter stabilization puzzle?

Session: 1. Environmental microbiology

Abstract:

The Arctic tundra and boreal forests store a major fraction of the global soil carbon. Most of this carbon is stored in the soil as soil organic matter (SOM). However, with the ongoing climate warming, Arctic and boreal ecosystems are at risk of turning from carbon sinks into net carbon sources.

Previously, the majority of stable SOM has been thought to originate from recalcitrant plant litter and chitin- and melanin-containing fungal residues. However, these have all been proven to be relatively easily decomposed, thus we lack a thorough explanation of the underlying mechanisms of SOM stabilization.

The Arctic tundra and boreal forest floor vegetation is dominated by ericoid plants that rely on mycorrhizas to ensure sufficient uptake of nutrients in the acidic and nutrient-poor environment. The soil is also abundant in plant secondary metabolites (PSM), namely condensed tannins (CT), which are known for their role in plant stress tolerance as well as their ability to precipitate proteins and other nitrogen-containing compounds, including a constituent of the fungal cell walls, chitin.

Preliminary results show that CT and fungal necromass (FNM) can form complexes in vitro that persist in the soil longer than the bulk of organic matter. By combining microbiological and biochemical methods, our study aims to extrapolate this mechanism across the arctic and boreal zone soil ecosystems, proving that complex formation between CT and FNM is a missing piece in the SOM stabilization puzzle.

Session 2. Molecular microbiology

Presenter: Khosrow Mohammadi

Title: Development of sporobeads coated with hEcad1/2 for rapid detection and capturing

Session: 2. Molecular microbiology

Abstract:

Internalin proteins localized at the surface of pathogenic Listeria monocytogenes interfere with E-cadherin to adhere and internalize into mammalian cells. E-cadherin has five extracellular, immunoglobulin-like domains (EC1 to EC5), of which the first domain is sufficient to mediate L. monocytogenes invasion. Immunomagnetic beads employ antibodies that react either with pathogenic or non-pathogenic Listeria. To obtain cost-effective and easy-to-produce beads for detecting pathogenic L. monocytogenes, we cloned and efficiently expressed human E-cadherin domains 1 and 2 (hEcad1/2) in the Bacillus subtilis spore coat. The cDNA sequence encoding hEC1/2 protein 23.7 kDa (MH511517.1) was inserted in pET22b and expressed and purified from Escherichia coli BL21. We used the CotY as a significant structural component of the B. subtilis spore coat to express hEcad1/2. We constructed a recombinant plasmid p1CSV-CotY-N-hEcad1/2 incorporating the cotY-hEcad1/2 gene under the control of the cotY promoter. The constructed plasmid was transformed into B. subtilis KO7 by double cross-over method and an amylase-inactivated mutant was generated. After spore induction, the developed sporobeads showed a high binding affinity for L. monocytogenes 4b. This result demonstrated the potential of human E-cadherin ectodomain 1 and 2 in a practical application involving the detection and capture of pathogenic L. monocytogenes.

Presenter: Veera Partanen

Title: The spread of antibiotic resistance genes in a microbial community

Session: 2. Molecular microbiology

Abstract:

Antimicrobial resistance (AMR) is one of the major challenges humanity faces in the near future. Already in 2019, the deaths of 5 million people were associated to resistant pathogens. Antimicrobial resistance genes (ARGs) can spread from harmless bacteria to pathogenic strains through horizontal gene transfer (HGT). Even one transfer event can make a strain multi-resistant and therefore problematic, even lethal. Previous studies suggest that, geographically, there's a positive correlation with warmer climate and higher antimicrobial resistance. However, from these real-world samples we can't separate the effect of temperature from other factors such as socio-economical ones that could affect the prevalence of resistance. Therefore, to study the effect of different temperature and antibiotic conditions on HGT of ARGs we conducted a time series microcosm experiment with a synthetic microbial community as receivers and E. coli as the donor. We added a sulfonamide resistance gene to a non-mobilizable plasmid on its own or in a mobile genetic element and added all the gene versions to the community in a live host or as extracted DNA. We followed the spread of the genes with Emulsion, Paired-Isolation and Concatenation PCR (epicPCR) and sequenced the products with PacBio to get long read data. Based on the initial results, the genes seem to have spread in the community and some species may be more likely to acquire the genes in some conditions. In my poster, I will show the first results from this experiment and discuss the next steps in our research plan.

Presenter: Dongming Zhang

Title: Revisiting *Aspergillus niger* Mst sugar transporters

Session: 2. Molecular microbiology

Abstract:

Aspergillus niger is one of the most studied plant-biomass-degrading filamentous fungus. In silico study reported an extensive set of putatively diverse sugar transporters (STs) in A. niger, which are predicted to play key roles in plant biomass conversion. However, the comprehensive understanding of STs diversity in A. niger is largely limited as only a few of them have been characterized. It is hypothesized that A. niger STs also have different affinities and overlapping specificities. We selected seven Mst transporter candidates from A. niger NRRL3, some of which have previously been shown or predicted to participate in glucose transport. To characterize them functionally, we used engineered Saccharomyces cerevisiae strain deficient in hexose transporters, disaccharide transporters and disaccharide hydrolases. Physiological characterization was performed through multiple A. niger Δmst strains engineered by CRISPR/Cas9 method. Expression of GreenFluorescentProtein-tagged Mst transporters showed that these transporters were localized to yeast plasma membrane. The preliminary growth analysis of the recombinant S. cerevisiae strains indicated that MstA, MstC, MstE, MstG and MstH are capable of transporting glucose and fructose, whereas MstD and MstF are not involved in the transport of these sugars. It also showed that MstE is an effective glucose ST with the previously characterized MstA, MstG and MstH. MstC has been classified as glucose ST based on expression, which is supported by our results. The multiple Δmst strains will reveal physiological contribution of these STs in A. niger. This study will shed light on the role of the Mst transporters and increase the possibilities to find novel glucose transporters in A. niger.

Session 3. Human / clinical microbiology

Presenter: Rasmus Malmgren

Title: Simulating aerosol transmission of respiratory viruses

Session: 3. Human / clinical microbiology

Abstract:

Respiratory viruses are a major cause of illness and reason for work absence. Many respiratory epidemics emerge seasonally at specific times of the year. To understand better what affects the seasonality of respiratory virus epidemics, we studied how IAV, CoV and RSV retain infectivity in different environmental conditions. We simulated real-life conditions by aerosolizing the viruses in a glass chamber where environmental conditions could be modified. In addition to virus infectivity, aerosol size distribution and concentration was monitored to control for changes in experiments with different environmental factors. By understanding the conditions where respiratory viruses thrive, we can better protect ourselves from future epidemics and pandemics and advance public health.

Presenter: Kuunsäde Mäenpää

Title: Skin bacteria of superficially wounded mice

Session: 3. Human / clinical microbiology

Abstract:

The skin microbiota during wound healing is a wide topic, and we explored the skin microbiota of superficial wounds for our upcoming studies, which included exposure to nanomaterials during wounding. We used hairless SKH-1 mice and divided them into two treatments groups. One group was treated with sterile PBS, and the other group was superficially wounded to simulate skin scratching and then treated with sterile PBS. Samples for transcriptome and microbiome analyses were collected 24 h and 7 d after treatment. At 24 h significant differentially expressed genes (DEGs) were related to immune response such as leukocyte recruitment, indicating the inflammatory stage of wound healing, while significant DEGs at 7 d were associated with cell signaling, suggesting the proliferative stage. These results on gene expression indicate the wound healing proceeded in an expected fashion. The skin microbiota communities were significantly different at 24 h but not anymore at 7 d. After 24 h of wounding notable changes in the relative abundance of amplicon sequence variants (ASVs) included a decrease of relative abundance of multiple Staphylococcus ASVs and an increase of ASVs such as Enterococcus. In a week, multiple Lachnospiraceae ASVs were increased in the wounded mice. These results will serve as a baseline for our future studies with nanomaterials and their possible use to benefit wound healing process in the skin.

Presenter: Rebecka Ventin-Holmberg

Title: Quantitative gut mucosal microbiota Inflammatory Bowel Disease during infliximab (IFX)

Session: 3. Human / clinical microbiology

Abstract:

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastro-intestinal tract growing rapidly in incidence and prevalence throughout the world and strongly associated with an imbalance of the gut microbiota. Moderate to severe IBD is treated successfully with anti-tumor necrosis factor alpha (TNF-α) infliximab (IFX) but up to half of the patients receiving IFX do not have a good long-term response. There are no methods available to predict the response to IFX, which would be crucial to save from both high costs and possible side-effects. Here the aim was to investigate the mucosal microbiota composition of the gut and to determine whether it differs in responders during IFX treatment. This was investigated in a cohort including 52 IBD patients from whom biopsy samples from both small- and large intestine were collected before, during and after infliximab treatment. The infliximab response was determined clinically and by colonoscopy at week 12 after start of treatment. The microbiota composition was determined by MiSeq sequencing targeting the 16S conserved rRNA from biopsy samples, and additionally absolute abundances were quantified by qPCR. The fecal microbiota composition is already published and is used here as comparison. Interestingly, the results of the mucosal bacterial microbiota show that there is a significant difference in the response group ($p < 0.05$), with SCFA-producing bacteria, particularly those in the class Clostridia, becoming more abundant during and after IFX treatment.

Presenter: Liina Hannula

Title: Potent SARS-CoV-2 inhibition by sherpabody TriSb92 - insights from cryo-EM

Session: 2. Molecular microbiology

Abstract:

The emergence of SARS-CoV-2 variants has caused concern for the efficacy of vaccines and therapeutics throughout the COVID-19 pandemic. To complement existing approaches of managing COVID-19, and to prepare for future outbreaks, it is necessary to develop broad-spectrum antiviral agents. A potential new class of virus inhibitors is sherpabodies, small (~7 kDa) protein binders engineered from the human nephrocystin SH3 domain. In this work, we describe a trimeric sherpabody TriSb92 that potently neutralizes a wide range of SARS-CoV-2 variants. To understand the neutralization mechanism and broad-spectrum activity of TriSb92, we used cryo-electron microscopy to study the inhibitor-bound SARS-CoV-2 spike protein. Interestingly, the inhibitor binding was associated with a conformational change in the spike, with all the flexible receptor-binding domains in the 'up' state. We postulate that this change is the first step in a larger conformational rearrangement that results in a nonfunctional spike, thus leading to virus inhibition. In addition, the TriSb92 epitope was revealed to be a cryptic site highly conserved across variants of concern, explaining the broad inhibition capacity. As the first example of an antiviral sherpabody, TriSb92 represents a powerful new approach to virus neutralization by small proteins.

Presenter: Annika Lintala

Title: Competition and accumulation of reptarenaviruses in co- and superinfections

Session: 2. Molecular microbiology

Abstract:

Boid inclusion body disease (BIBD) is caused by reptarenaviruses that destroys snakes' life quality leading eventually to death. A snake with BIBD usually carries more than a single pair of genetically distinct reptarenavirus small (S) and large (L) segments, and variation of S and L segment species is found within the tissues. We hypothesized that coinfection would only occur if the infecting viruses were genetically distantly related as seen previously with mammarenavirus studies. We employed boa constrictor kidney-

and brain-derived cell cultures (I/1Ki and V/4Br) for performing a set of co- and superinfection experiments with five reptarenavirus and one hartmanivirus isolates. We used qRT-PCR to follow the replication of each virus S and L segment in co- and superinfection to be compared to single infection. Released RNA from coinfected cells did not demonstrate any competition between reptarenaviruses in the I/1Ki cells. The experiments on V/4Br cells revealed considerable differences in the replication ability, suggesting varying tissue specificity for reptarenavirus species. Experiments on persistently reptarenavirus infected cell lines showed reduced replication of closely related reptarenaviruses while the replication of reptarenaviruses from different species appeared unaffected. The results provide supporting cell culture evidence to the hypothesis that consecutive reptarenavirus superinfections among snake populations has led to the accumulation of genetically divergent reptarenavirus S and L segments. Tissue tropism-like behavior was seen in the cell cultures and studying of it ought to be continued as well as the virions' RNA compositions, since they might have implications to the spread and pathogenesis of BIBD.

Presenter: Angélica de Lima das Chagas

Title: Klebsiella pneumoniae in co-infection with Covid-19 patients: systematic review

Session: 3. Human / clinical microbiology

Abstract:

Bacterial infections are one of the main complications of the clinical symptoms of hospitalized patients. They increase morbidity and mortality among patients as well as the costs associated with healthcare. The prevalence of patients hospitalized with Covid-19 and co-infected by pathogenic bacteria is relevant, considering the scope of treatment. This work is a systematic review that assesses the prevalence of Klebsiella pneumonia in co-infection with patients hospitalized for SARS-CoV-2, applying PRISMA guidelines. The results of the PubMed, Embase, SciELO, SCOPUS, and Web of Science databases were screened by selecting articles published in English, from December 2019 to October 2022. A total of 408 publications were found, of which 50 were eligible, and 35 were included in this study. The highest rate of bacterial co-infection by K. pneumoniae and SARS-CoV-2 was described in the Asian continent (23%, 624 out of 2,481 patients), and the ratio was 0.23 [0.14 to 0.35]. Regarding all the cases of co-infection reported in selected publications 8,741 hospitalized patients had bacterial co-infection and SARS-CoV-2 and K. pneumoniae represented 1,422 cases (19%). The co-infection described in this study is relevant due to its history of antimicrobial resistance and nosocomial infections highlighting the need for combined therapy in most cases. If a diagnosis of co-infection is not correctly performed, it will result in a poor human prognosis and inadequate treatment.

Presenter: Celia Regina Malveste Ito

Title: Viral co-infection in children with severe respiratory infection during Covid-19

Session: 3. Human / clinical microbiology

Abstract:

Respiratory viruses can cause severe infections in children. Samples of nasopharyngeal swabs of children with a diagnosis of Severe Acute Respiratory Infection were collected from children admitted to the Pediatric Intensive Care Unit, in hospitals in Brazil and, tested by polymerase chain reaction using specific oligonucleotides for respiratory viral. Results: A total of 446 children were infected with a single virus type and 160 were co-infected with two or more virus types. Twenty-two patients had SARI-causing viruses. The most frequent co-infections identified in the study were: Rhinovirus (RV)/SARS-CoV-2 (17.9%) and RV/Respiratory Syncytial virus (RSV) (14.2%). Among all those infected, the most prevalent age group was between 24 and 59 months (38.1%) of the patients. Patients older than 59 months (about 5 years) represented a total of 27.5%. The use of oxygen therapy was statistically significantly related to co-infections of BoV, RSV, Metapneumovirus, and another Coronavirus. In the year 2020, RV/BoV were more frequent in relation to other types of coinfections, representing a total of 35.1%. In 2021 presented a divergent profile, with RV/SARS-CoV-2 coinfection being the most frequent (30.8%), followed by RV/RSV (28.2%). Additionally, 25.6% and 15.4% represented coinfections between RSV /SARS-CoV-2 and RV/AdV, respectively. Conclusion: Co-infections with respiratory viruses, such as RSV and BoV, can increase the severity of the disease in children with SARI who are admitted to the PICU, and children infected with SARS-CoV-2 have their clinical condition worsened when they have comorbidities.