Wednesday 20 September

Microbes in Health and Disease

In vitro technology to study live biotherapeutics and enteropathogens in the human gut

Keynote speaker: Dr. ir. Kim De Paepe
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Nearly 2500 years ago, Hippocrates emphasized the central role of the human gut in host health, stating that “death sits in the bowels” and a “bad digestion is the root of all evil”. Traditional microbiology supported these statements by unravelling the role of pathogenic gut micro-organisms in infectious diseases. Although infectious diseases are in decline, causing just 2.6% of all deaths globally, Hippocrates’ claims still hold true today as chronic, non-communicable diseases such as obesity are the leading cause (74%) of death. These so-called ‘Western’ diseases are associated with a disturbed interaction between micro-organisms, host and environment. This multifactorial etiology makes it difficult to move past associations and reach a mechanistic understanding of how the impaired interactions cause debilitating conditions. Adding to that, host micro-organisms are genetically and functionally divers, highly individual and inaccessible, the *in vivo* host is difficult to experiment with and the environment is hard to control. *In vitro* platforms ranging from batch and fed-batch fermentations to dynamic semi-continuous simulators have, therefore, been devised to mimic the host microbiota. Besides microbial endpoints, markers of host health, such as epithelial barrier integrity and immune homeostasis can be examined *in vitro* through co-culturing of human epithelial and macrophage like cells followed by exposure to host microbiota samples. This integrated *in vitro* workflow permits in-depth mode-of-action studies of micro-organisms in health and disease by enabling frequent sampling at a high spatial resolution, without ethical constraints at a high level of control, abstracting possible confounding effects due to the background diet/lifestyle or participant non-adherence. This approach will be illustrated with a case study on *Akkermansia muciniphila*, a micro-organism that is put forward as a live biotherapeutic to abate the globesity (global obesity) epidemic. *In vitro* models are also useful to study colonization by enteropathogens such as ETEC in the context of infectious diseases. Finally, personalized *in vitro* research will be discussed as a stepping stone to a more targeted personalized treatment that fits well in the personalized medicine framework that is required to lower the burden of non-communicable diseases on global healthcare systems.

Towards standardization of next-generation real-time bioaerosol monitors

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Bioaerosols, comprising diverse organic particles from various ecosystems, exert significant influences on human health, agriculture, and natural environments. Recent advances in technology have facilitated real-time measurement of airborne biological particles through automated monitors, coupled with cutting-edge machine learning capabilities. Detecting and characterizing these aerosols have gained momentum with various techniques, ranging from impactors with digital microscopes to airflow cytometers that use fluorescence spectroscopy and scattered light.

However, the absence of established standards for this novel class of instruments poses challenges. As this field matures, the transition from the standard manual method to automated systems warrants meticulous standardization in all aspects of the measurement chain, spanning measurement calibration and algorithmic development. The measurement accuracy of bioaerosol properties, whether they be physical or chemical, should be assured through systematic calibrations against a reference, necessitating the availability of meticulously labelled reference bioparticles. Yet, the uniqueness and complexity of bioaerosols, encompassing pollen and fungal spores, lie in their intricate morphological and chemical variability.

A key prerequisite for calibrating next-generation instruments and training identification algorithms is the availability of accurately labeled reference data. Culture collections, as repositories of diverse micro-organisms, may fill the gaps and be pivotal facilitators by supplying well-characterized reference material that are crucial to refine measurement accuracy and algorithm robustness. Collaborative efforts between the aerobiology community and culture collections should offer a promising avenue for ensuring the future quality, reliability and comparability of bioaerosol measurement results.

Is hand disinfectant a public health problem? A case study in the Centre of Biological Engineering

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For about three years, the world was faced with the CoVID-19 pandemic that showed us the threats of unknown microorganisms. At the beginning of the pandemic several behavioural rules for prevention and protection were imposed. Besides the use of masks, ethanol solutions for hand disinfection were one of the most common measures. Public recipients of alcohol were seen and accessible in every public and private institutions and commercial places. However, with the official end of the pandemic this year (and even before that), it was possible to observe the abandoning of these recipients. Some stay in place empty and others still with the ethanol-glycerol solution.
In the Centre of Biological Engineering building, we came to notice that some of these recipients were colonized with fungi. The aim of this work was to collect the fungal colonies and try to assess which fungi are present and their taxonomical variation.
Fugal colonies were collected from 4 different recipients. The number of isolates from each recipient ranged from 2 to 10. Based on morphological characteristics it was possible to identify some of the isolates as Aspergillus sp., Penicillium sp. and Cladosporium sp. To complement this information molecular identification is being carried out to try to get identification at species level.
We acknowledge that culture collections have a leading role in the search and preservation of microorganisms as well as public warning of their potential risks. Consequently, we intend to expand the sampling spots and make recommendations to the university staff (and the population in general) to assure that this is not a potential public health problem.
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The oral cavity: a mouthful of opportunities

Keynote speaker: Joossens Marie
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The oral cavity is an important gateway for bacteria to the gastrointestinal and respiratory tracts. The ecological community in the oral cavity of humans contains more than 700 bacterial species, including at least 11 bacterial phyla and 70 genera1. It is believed that each individual carries more than 200 species. In addition to the very different bacteria that colonize the oral cavity, many more pass as temporary members of the oral microbial community.
As with the gut microbiome, oral microbiome compositions are also suggested to play a role in human pathophysiology. Because sampling is perceived as less unpleasant by participants, standardization is feasible, and animal research supports a causal role of oral bacteria in various diseases, the potential use of oral bacteria is currently underexplored in human health and disease. Stimulated by breakthroughs and knowledge about bottlenecks in gut microbiome research, a selection of the most recent insights into the links between systemic diseases and the oral microbiome is presented.

Dewhirst, F. E. et al. The human oral microbiome. J. Bacteriol. 192, 5002–17 (2010).

The VirusBank Platform: A new and unique research infrastructure enhancing preparedness against future epi- or pandemics

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The global impact of the SARS-CoV-2 pandemic has revealed deficiencies in health surveillance, disease prevention, and societal readiness against pathogens with epi- or pandemic potential. Investing in pandemic preparedness is crucial to address the global burden of infectious diseases. Accordingly, to promote such preparedness, the Belgian Federal Government has invested in establishing a 'VirusBank Platform' at the University of Leuven (KU Leuven).
The mission of the VirusBank Platform is to develop a state-of-the-art assay toolbox and infrastructure to expedite the development of prophylactic or therapeutic biopharmaceuticals (vaccines, antibodies, and antiviral drugs) against viruses belonging to viral families with high epi- or pandemic potential (www.virusbankplatform.be). The VirusBank Platform experts specifically aim to: 1) establish a strategic collection of viruses representing an epi- or pandemic concern; 2) develop a toolbox of cell culture models using (reporter) cell lines, human primary cells, and organoids; 3) create multiplex assays for high-throughput antiviral screening; 4) establish virus infection models in rodents and zebrafish larvae; and 5) use libraries of small molecules to perform compound screening campaigns.
To support these goals, the VirusBank Platform combines and centralizes relevant viral expertise, and provides a highly secured and unique research infrastructure consisting of a brand-new biosafety level 2 (BSL-2) facility fully equipped with automated, high-throughput research equipment, and facilities for working with zebrafish larvae, organoids, and primary cell lines. The VirusBank Platform is also associated with the Rega Institute, the Neyts Lab (www.antivirals.be), and CD3 (www.cd3.be), which offer BSL3 cellular and BSL2/3 animal laboratories, including its Caps-It research infrastructure

(https://rega.kuleuven.be/cmt/capsit). CD3 provides a fully automated compound handling platform, while the Caps-It core consists of an automated platform for high-content imaging analysis of live pathogens of high or unknown biosafety risk.
The VirusBank Platform altogether goes beyond being just a virus collection. It strives to offer an end-to-end platform for research on virus families of epi- or pandemic concern, thereby facilitating the development of new preventive or therapeutic strategies against these viruses.

Fungi from marine plastisphere as source of osteogenic and mineralogenic compounds

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Osteoporosis and osteopenia are human chronic disorders characterized by low bone mineral density. Bisphosphonates have anti-resorptive properties and are commonly used to treat osteoporosis. However, they are associated with rare and acute side effects, thus the discovery of alternative drugs is highly relevant to the field. The search for novel anti-resorptive and/or bone anabolic compounds has gained momentum in the last decade and marine environment was found to be rich in osteo-active molecules, probably because marine organisms are subjected to extreme conditions fueling specialization and adaptation. Among organisms that have already demonstrated a remarkable diversity of bioactive compounds, fungi appear to have the potential to synthetize primary and secondary metabolites with osteogenic activity. In this work, we explored the biopotential of marine fungi (both filamentous and yeasts) associated to marine microplastics (MPs). MPs were sampled in the sediments of three sites of the Tyrrhenian Sea characterized by different degrees of anthropic impact (the harbor of Livorno, the Marine Protected Area "Secche della Meloria" (MPA) and an intermediate area of the transept), using a culturomic approach. Fungi were cultured at 15 °C on solid media with different degrees of oligotrophy for 4 weeks and identified through the molecular analysis of specific markers and the evaluation of morpho-physiological characteristics. A total of 60 fungal strains (17 taxa) were isolated from MPs; most of them are Ascomycota, while about 30% of the remaining are Basidiomycota (exclusively yeasts).
A selection of 17 strains (one strain per taxon) were screened for the presence of bioactive compounds to be used in the treatment of low bone density chronic disease. Fungal biomass was prepared from liquid fermentation (using PDB broth) and solid fermentation (using rice) to stimulate the production of different bioactives, and 34 extracts were produced using ethyl acetate. Extract bioactivity was evaluated in vitro and in vivo using bone-derived cell lines capable of extracellular mineralization to assess their mineralogenic potential and developing larval zebrafish operculum to assess for their osteogenic potential, respectively.
Results showed that extracts from three fungal strains (Aspergillus jensenii from liquid fermentation; Cladosporium halotolerans and Penicillium brevicompactum from solid fermentation) significantly increased opercular bone growth in vivo. Five extracts (Aspergillus jensenii and Penicillium bialowense from liquid fermentation; Cladosporium cladosporioides, Sesquicillium microsporum and Cladosporium pseudocladosporioides from solid fermentation) increased extracellular matrix mineralization in vitro. Extract prepared from liquid fermentation of Aspergillus jensenii was the only one presenting activity in vivo and in vitro. Therefore, it was further analyzed through High Performance Liquid Chromatography and Nuclear Magnetic Resonance coupled with mass spectrometry. Results revealed the presence of large quantities of Decumbenone A and

Decumbenone B, which were purified and will be further characterized for osteogenic and mineralogenic activity.
Our findings indicate that crude extracts from marine mycobiota of the plastisphere are potential sources of osteo-active molecules with possible therapeutic applications in the treatment of human bone disorders.

Using a polyphasic approach for exploring secondary metabolites from freshwater and thermal cyanobacteria strains from Azores islands

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The BACA culture collection (Bank of Algae and Cyanobacteria of the Azores), created in 2018 in the University of the Azores, is a ECCO member as well as a PT-mBRCN member and holds more than 800 strains of microalgae and cyanobacteria, all isolated from the Azores archipelago. BACA currently holds 811 strains, 406 cyanobacteria, 318 green microalgae, 85 diatoms, and one dinoflagellate, with more than 600 strains genetically characterized, with strains from the nine islands of the Archipelago, and all available habitats in the archipelago (e.g. freshwater, brackish and marine lakes, terrestrial, thermal). The taxonomical revision of the cultured strains, using morphological, ecological, and genetic data, allowed the report of a unique cyanobacteria diversity in the collection and the Azores, with several strains in the BACA collection to be described as new taxa. The collection has been screened for the presence of cyanotoxin producing strains, with the report of several cyanobacteria species producers of toxins such as microcystin, saxitoxin, cylindrospermopsin and anatoxin, some of them undescribed toxin producers. These strains are isolated from several different habitats (both freshwater and brackish), from several Azorean islands, revealing a high distribution and possible impact in the ecology of the Azorean aquatic systems. These results highlight the need to promote and support culture collections, such as BACA, that have contributed to the knowledge of diversity, toxicology, and biotechnological applications of algae present in the Azores, allowing a better understanding of the opportunities and problems that might come from the presence of such taxa in the Azores.

Microbes in Health and Disease

Biocontrol of plant pathogens combining AMF and bacteria

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B. velezensis (referred as Bv here below) is a typical soil dwelling plant-associated bacterial species that retains a high potential to protect crops against diseases and therefore represent a promising alternative to chemical pesticides as biocontrol agent. The two main modes of action for biocontrol are direct antagonistic activity toward pathogens and the ability to trigger an immune reaction in the host plant leading to a systemically-expressed higher resistance to subsequent infection (induced systemic resistance or ISR). These functionalities are mainly mediated by some secreted bioactive secondary metabolites (BSMs) also referred as specialized metabolites. This chemical arsenal includes a wide range of metabolites but how and to what extent their production may be modulated upon interaction with other microbes sharing the niche or with the host plant is not well known. In that context, cross-kingdom interactions between Bv and soilborne fungi usually result in antagonistic outcomes and fungal growth inhibition due to efficient production of toxic lipopeptides by the bacterium. In a recent work, we unveiled a rather unanticipated compatibility between this strong bacterial competitor and the arbuscular mycorrhizal fungus Rhizophagus irregularis (R.i.) as another keystone microbial species providing benefits to plants. Our findings describe how compatibility between these two keystone species is mediated via taming BSM expression in the bacterium. Such compatibility ensures stable coexistence and partnership with reciprocal benefits for the two microbes but also for the host plant in terms of protection against pathogen infection via ISR.

Microfluidics to study microorganisms at the cellular level

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First developed for chemical synthesis and analysis, microfluidic (or Lab-on-a-Chip) technology, quickly
outgrew its origins and has, in recent years, become widely established as a useful tool for biological applications. Microfluidic platforms generally involve microchannels containing either a continuous gas or liquid phase, or a droplet-based system. The miniaturisation achieved with these microfluidic platforms allows for an enhanced controllability of system processes, like chemical reactions or incubation, as well as higher analytical accuracy and sensitivity, while the enclosed nature of microfluidic devices entails an intrinsic sterility and containment of pathogens or hazardous substances. For the study of (micro)organisms, microfluidic technology offers optical transparency for precise brightfield or fluorescence imaging and the ability to mimic aspects of the natural habitat, including physical shapes and obstacles as well as chemical gradients. These benefits have already been recognised and broadly utilised by numerous researchers for organisms from all five natural kingdoms. For instance, microfluidic technology is aiding to uncover mechanisms regarding the establishment and interactions of beneficial microorganisms, which has great importance for agriculture. Moreover, infection mechanisms of pathogens, of either medical or agricultural interest, can begin to be unravelled. Microfluidic technologies are also used to extract target organisms from complex samples, as well as sort them, subject them to stimuli and analyse their behaviour. Studies have revealed, for example, intricate space searching strategies in soil-dwellers such as the plant

symbiont mycorrhizal fungi, as well as how fungi and bacteria interact to explore the microcosm, events that normally occur inside a “black-box” and are thus hidden by the opacity of soil. As the field of microfluidics is finding its way into mainstream lab practices, more knowledge gaps will be filled and intriguing findings acquired.

Heterogeneity in food spoilage fungi

Keynote speaker: Jos Houbraken
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Mild preservation protocols, often in combination with controlling fungal contamination, are utilized to combat fungal spoilage. Ideally, a preservation system should be effective against all spoilage fungi, but most (novel) preservation protocols tend to be species specific. Besides this interspecific variation, also intraspecific variation occurs, and food preservation becomes even more challenging when considering intra-strain variation, e.g., the heterogeneous character of conidia in their stress resistance and germination capacity. This inter- and intraspecific variation also impacts taxonomic studies Accurate identification remains important for effective communication and recognition of unique properties and traits associated with specific fungal species. While species delimitation appears to be clear-cut, studies in Aspergillus reveal that species boundaries become more robust and accurate with an increased understanding of variability. When including this variability in taxonomic studies, new species are discovered, but also known food spoilage species changed name. In this presentation, the extent of heterogeneity at the inter- and intraspecific level, in relation of food spoilage and taxonomy, will be discussed. Needlessly to say, strain collections play a crucial role in these studies.

Yeast diversity of cocoa bean heap fermentations and their environments

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The raw material for chocolate manufacture are fermented dry cocoa beans. The cocoa bean fermentation is performed locally at farms or cooperatives in a largely uncontrolled process. In contradiction with the product value and the increasing demand for high-quality chocolate, post-harvest processing of cocoa is not optimal in many cases.
Cocoa pods grow on the trunk and branches of the tropical cocoa tree (Theobroma cacao) after pollination of the flowers by Forcipomyia midges. To recover the commercialized beans, the harvested pods are opened and the beans, which are embedded in a mucilaginous pulp, are extracted. During its removal the pulp-bean mass is massively contaminated by environmental microorganisms. This ‘inoculation’ is in addition to a far lower load of micoorganisms that may have entered cocoa pods before harvest. The resulting mixed fermentation by lactic acid bacteria and yeasts liquefies the pulp, which drains away, and produces a complex set of metabolites that influence the quality and taste potential of the cocoa beans. Inoculated test fermentations resulted in generally similar qualities compared to the traditional spontaneous process. Such inoculated fermentations reinforced interest in yeasts for their essential role in the production of chocolate. The yeasts that can be isolated from the environment of the cocoa bean fermentations are highly diverse, while the fermentations are dominated by the genera Hanseniaspora, Saccharomyces, and Pichia. The numerous yeast diversity studies of cocoa bean fermentations in the main cocoa growing areas of South America/Caribbean, Africa, and South East Asia make it interesting to compare the yeast species diversity in relation to the geographic location to search of evidence for the source of the main fermentation species.

Lactobacilli and yeasts for phytate degradation in soy milk fermented products

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Due to the increasing prevalence of dairy intolerance and the adoption of vegan/vegetarian diets, there is a growing demand for non-dairy alternatives. One commonly used substitute in vegetarian and vegan food products is soybean, which provides minerals and proteins. However, soybean also contains the antinutrient phytate (inositol hexaphosphate, IP6), which hinders nutrient absorption. To address this, microbial phytases, which are enzymes hydrolyzing phosphate groups from IP6, have gained significant attention. Degradation of IP6 releases chelated minerals, such as Fe and Zn, and solubilize IP6, resulting in increased mineral availability for intestinal absorption.
The aim of the present study was to investigate 10 Lactic Acid Bacteria belonging to Lacticaseibacillus casei, Lactobacillus delbruecki, L. helveticus, and Lactiplantibacillus plantarum, and 22 yeasts, previously isolated from Tajikistan yogurt and togwa (Tanzanian traditional fermented cereal- and cassava-based product) for i) fermentation capacity (singularly or in mixed cultures) of soy milk and ii) phytase activity. All strains belonging to Lacticaseibacillus casei showed the ability to ferment soy milk and thicken the product. With respect to the yeasts, Kluvieromyces marxianus and Pichia kudriavzevii were selected. The previously observed strong phytate degrading ability of strain P. kudriavzevii TY1322 in vitro trials was confirmed in this study during soymilk fermentation. The phytate degradation was further improved when strain P. kudriavzevii TY1322 was used in co-culture with Lactobacillus casei AB3 and K. marxianus AL3 or BL8 suggesting these strains as promising candidates for phytate degradation in soy-based food fermentation.

Thursday 21 September

Microorganisms in ecological applications

Understanding the mechanisms of stress resistance to optimize the application of cyanobacteria for soil restoration

Keynote speaker: Beatriz Roncero Ramos
University of Seville, Spain

Drylands cover around the 40 % of the Earth's surface and host more than one-third of the world's population. They are facing an increasing soil degradation due to climate change and land-use intensification; hence, their restoration is more necessary than ever. However, their recovery by traditional methods based on plant establishment is not successful, due to the water limitation in these ecosystems. Thus, specific restoration methods adapted to the strong environmental stress factors in drylands are being developed. One of them is the cyanobacterial inoculation of soil to recover the biological soil crusts or biocrusts. These communities colonize soil surfaces in drylands and are composed by fungi, lichens, mosses, cyanobacteria, archaea, other heterotrophic bacteria and microalgae. Biocrusts provide multiple ecosystem services, such as: increasing soil fertility, decreasing soil erosion or regulating the soil water balance. Cyanobacteria are the first colonizers of soils and participate in the first stages of biocrust succession. Many studies have already proved the ability of different cyanobacterial species to induce the formation of artificial biocrusts after inoculation on several soil types. However, field inoculation studies have shown that the capacity of cultured cyanobacteria to survive under natural conditions needs to be optimized. One strategy is to harden cultures before inoculation to be re-adapted to extreme field conditions. Thus, we have started applying multiple stress factors to cyanobacteria to trigger the resistance mechanisms they have and analysing their physiological response. We are combining these results with genomic and transcriptomic data. This information will allow us designing an efficient hardening technique for cyanobacterial field inoculation in drylands. Here, we show the physiological response of an Antarctic cyanobacterium under multiple stress factors, as well as its genome annotation to analyse its resistance mechanisms.

Harnessing local rhizobia for soybean cultivation at northern latitudes

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Over 90% of the soybean used in the European Union is imported, endangering vulnerable ecosystems, especially in South America. Increased local production of protein-rich soybean plants would make consumption more sustainable. Early-maturing soybean varieties have already been cultivated and screened to cope with the colder climate. As a legume crop, soybean engages in a symbiosis with rhizobia bacteria that they host in root nodules and that supply the plants with fixed nitrogen. However, the available commercial inoculants contain non-endemic strains and are not adapted to the North-West European conditions, resulting in suboptimal yields. Therefore, the citizen science project 'Soybean in 1000 Gardens', a large-scale trapping experiment, was set up in Flanders to acquire indigenous rhizobial strains able to efficiently nodulate soybean. Root nodules were harvested and cut in half: one half was used for microbiome analysis and the other half for isolating strains. This gave insight into the identities and abundances of the bacterial strains residing inside the

root nodules while also building an isolate collection comprising currently over 600 strains belonging to 68 genera. Plant confirmation experiments were performed to test the nodulation capacity of the rhizobial strains. Whole-genome sequencing of strains belonging to the genera Bradyrhizobium, Rhizobium, and Tardiphaga allowed a better understanding of the potential of these strains as inoculants. So far, three Bradyrhizobium strains were found to effectively nodulate soybean and are currently being tested in a second field trial.

CoSMi microalgal collection at OGS: Exploring the phytoplankton potential in the circular bioeconomy

Manuela Bordiga
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Cultivating phytoplankton retains a huge potential for carbon (C) sequestration and biomass exploitation. Phytoplankton is capable to adsorb variable amounts of CO2 through photosynthesis transforming the inorganic C into organic C. Moreover, the production of species-specific exoskeletons composed by bioproducts (e.g. calcium carbonate, silicate), reusable within the circular bioeconomy, makes this group a great resource for the Carbon Capture Usage and Storage (CCUS). A rich microalgal collection represents a valuable source for identifying the best strains and testing the conditions for their exploitation. At the National Institute of Oceanography and Applied Geophysics (OGS, Trieste, Italy), the Collection of Sea Microorganisms (CoSMi), linked with international research infrastructures such as MIRRI.IT, LifeWatch, ECCSEL and EMBRC ERIC, is maintained. CoSMi retains around 100 marine microalgal strains including diatoms, dinoflagellates, coccolithophores and some classes of flagellates mainly from the Adriatic Sea. CoSMi, thanks to its collection, specialized researchers and technicians, and numerous facilities and services for cell cultivation and experimentation such as climatic chambers, high-tech photobioreactors, minicosms, chemical and genetic analyses, allows testing the best culture conditions to maximize the biomass production for future exploitation. Moreover, in the framework of the Italian PNRR project, SUS-MIRRI.IT, CoSMi collection will be further strengthened in the next two years. In the past, at OGS some studies have been conducted on the potential of specific strains of chlorophytes and coccolithophores in the biofuel production. Currently, we are focusing on the use of coccolithophores in the CCUS, by studying their response towards increasing CO2, elevated nutrient conditions, and resilience to wastewater. Coccolithophore bioproducts may be reused as paint whitening or cement components, fertilizers, nutraceutical, or even in nanotechnology.

Running a photobioreactor in space for the production of oxygen and edible 'spirulina' biomass

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Microbially produced oxygen and microbial edible biomass are very interesting sustainable resources for future space travelers. Arthrospira-C (ArtC) is a space biotechnology flight experiment, to transplant cyanobacterial oxygenic photosynthetic bioprocess to space.
ArtC is the follow-up and step-up of the pioneering ArtB flight experiment which flew in Dec. 2017 to ISS, this time allowing continuous culturing and variariable light settings. A space compatible photobioreactor was built, allowing online measurements of both bio-oxygen production rate and microbial growth rate in space. They contain the cyanobacterium Limnospira indica PCC8005 (aka Arthrospira sp. PCC8005, or under the product name 'spirulina'). Four of such bioreactors are to be integrated in the ISS Biolab incubator in 2023 and will be operated in turbidistat mode by contineous feeding for a duration of 2 months, to test production kinetics at 4 different light settings. The experiment will be performed in parallel on ISS and on ground. The bioprocess will be monitored and steered, using a novel and dedicated model for the growth of Limnospira in membrane photobioreactors, and the space grown biomass will be analysed for its nutritive value in detail postflight.
In this presentation we will update you on the current development of the ArtC flight experiment and we will adress following challenges:
(1) to build, qualify, and operate a turbidistat photobioreactor in space, with special attention to liquid mixing and CFD modelling of reactor designs and operation states
(2) to restart the cyanobacterial cultures in the bioreactor in ISS after a period of storage and upload,
(3) to maintain an axenic photosynthetic active culture in the bioreactor, under space conditions,
(5) to monitor online the oxygen and biomass production of the culture, and the fitting to the predictive simulation, and
(4) to implement successfully ground commands to adapt bioreactor conditions and allow several crew interventions.
In addition, some reference data obtained pre-flight on ground will be presented and discussed. A good fitting was achieved between the predictive simulation and the experimental results obtained for oxygen and biomass production in the space bioractor, when a good mixing of the liquid phase was maintained. The pigment and proteomic profiles of the biomass confirmed full activation of the photosynthetic cellular processes, in the reactor conditions on ground.
These data show it is feasible to design and operate a space-compatible continuous microbial photobioreactor, which is ready to be tested in the International Space Station.

Keywords : MELiSSA, photobiobioreactor, flight experiment, Limnospira, Arthrospira, model.

Diversifying application of recycled nutrients derived from anaerobic digestion for sustainable algal protein farming

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The climate change-induced stress on conventional protein sources necessitates the exploration of sustainable alternatives to supplement increasing global nutritional demand. Microalgae, capable of producing value-added biomass (40-70% w/w DM protein content), utilizing recycled nutrients and acclimatizing to (environmental) stress conditions, are being considered as a promising feedstock for large-scale algal protein production. This research focuses on the integration of phototrophic microalgal production and anaerobic digestion to generate protein-rich (upto 67% w/w) biomass using recycled nutrients. The preliminary experiments investigated the pre-treatment of food waste-based digestate for cultivating Desmodesmus sp. and Chlorella vulgaris. Paper-filtered digestate was found to improve microalgal growth compared to commonly used membrane-filtered digestate using microplate-based (3 mL) screening methodology coupled with Cytation device (image sensing) assessment. However, phosphorus supplementation was necessary to achieve enhanced growth, emphasizing the need for balanced growth medium and additional processing/pre-treatment when utilizing recycled nutrients. Ammonium sulphate (ASD), another digestate-derived nutrient was used as a nitrogen source for cultivating Desmodesmus sp. The obtained results from lab- (3 L) and pilot-scale (550 L) photobioreactor studies presented that ASD could potentially replace mineral nitrogen sources, reducing the operational costs and contributing towards a fully circular 'protein' economy. Furthermore, the optimization of abiotic process conditions for microalgal growth was addressed through a dynamic mathematical model, enabling the prediction of Desmodesmus sp. growth in a large-scale reactor. The key performance indicators considered were biomass growth, protein concentration within biomass and nutrient concentration in effluent. The model indicated that nitrogen origin didn't strongly influence the protein yield, supporting the use of nitrogen derived from digestate. Finally, extended assessment of different food/feed-relevant microalgal species (including Kirchneriella sp.) for their temperature tolerance (23 – 40 ˚ C) was done in microplates. Desmodesmus sp. showcased resilience to higher temperatures (~32.5 ˚ C) and successfully grew in digestate, underlining the potential of microalgae as a sustainable high protein (57% w/w) source. This research finally indicates the potential of microalgae as a viable and sustainable alternative for protein production, leveraging the integration of algal production and anaerobic digestion to address the impending global protein demand and food/feed insecurity.

Keywords
climate change; digestate; microalgae; microplate experiments; photobioreactor; protein ; recycled nutrients

A phylogenomic and comparative genomic analysis of Commensalibacter, a versatile insect symbiont

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Background: To understand mechanisms of adaptation and plasticity of pollinators and other insects a better understanding of diversity and function of their key symbionts is required. Commensalibacter is a genus of acetic acid bacterial symbionts in the gut of honey bees and other insect species, yet little information is available on the diversity and function of Commensalibacter bacteria. In the present study, whole-genome sequences of 12 Commensalibacter isolates from bumble bees, butterflies, Asian hornets and rowan berries were determined, and publicly available genome assemblies of 14 Commensalibacter strains were used in a phylogenomic and comparative genomic analysis.

Results: The phylogenomic analysis revealed that the 26 Commensalibacter isolates represented four species, i.e. Commensalibacter intestini and three novel species for which we propose the names Commensalibacter melissae sp. nov., Commensalibacter communis sp. nov. and Commensalibacter papalotli sp. nov.. Comparative genomic analysis revealed that the four Commensalibacter species had similar genetic pathways for central metabolism characterized by a complete tricarboxylic acid cycle and pentose phosphate pathway, but their genomes differed in size, G + C content, amino acid metabolism and carbohydrate-utilizing enzymes. The reduced genome size, the large number of species-specific gene clusters, and the small number of gene clusters shared between C. melissae and other Commensalibacter species suggested a unique evolutionary process in C. melissae, the Western honey bee symbiont

Conclusion: The genus Commensalibacter is a widely distributed insect symbiont that consists of multiple species, each contributing in a species specific manner to the physiology of the holobiont host.

Taxonomy, Phylogeny, Phylogenomics, Phylodynamics: new techniques and approaches

Using genomes for the classification and tracking of mycobacteria

Keynote speaker: Conor J. Meehan
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The genus Mycobacterium has been described for over 100 years and contains a multitude of both rapid and slow growing bacteria, including several important human pathogens. Recently, this genus was split into 5 genera based upon phylogenetic monophyly, average amino acid identity and signature conserved indels and proteins. However, the support and need for this division has been debated since.
Although species definitions based upon genomic content (e.g. using the average nucleotide identity) are well known, molecular definitions of a genus are not as concrete. We applied a variety of genome-based tools for defining a genus, all of which support the original Mycobacterium genus grouping. These findings combined with the clinical and historical factors that weigh heavily on renaming such an important genus suggest that the new division into five genera should be reversed and the original designation of Mycobacterium should be applied to all such species.
In a similar vein, transmission dynamics of pathogens based upon genomic content (e.g. SNP cut-offs and genome similarities) are well known, but for mycobacteria can be insufficient due to the high similarities between strains of the same species. We used a variety of phylodynamic approaches to track transmission of Mycobacterium tuberculosis in Rwanda and The Gambia, combining genome sequences with Bayesian phylogenetic approaches to better resolve transmission patterns and assess the impact of public health intervention efforts. As more genomes are gathered worldwide for mycobacterial species, these phylodynamic methodologies will allow us to better understand infection patterns and reduce ongoing transmission.

Dermatophytes fungi: Phylogenomics of the Trichophyton mentagrophytes species complex

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Sciensano, Belgium

The Trichophyton mentagrophytes complex is a group of pathogenic fungi causing dermatophyte infections in both animals and humans. The taxonomy and phylogeny of this complex have been a subject of debate due to their phenotypical similarities and genetic overlaps.

Our research focuses on the T. mentagrophytes and T. interdigitale species, for which 28 different ITS genotypes have been described. Of particular interest is T. mentagrophytes type VIII, recently classified as a new species, T. indotinae. This genotype has caused a widespread, therapy-resistant outbreak of dermatomycosis in India, replacing T. rubrum as the main pathogen responsible for skin infections.
The increase in T. indotinae infections is linked to mutations in the SQLE gene, rendering the strains resistant to terbinafine treatment.

Species identification based on clinical characteristics and the source of isolation and the constantly shifting nomenclature has caused confusion when identifying strains belonging to the T. mentagrophytes complex. This is also reflected in the often incorrectly named sequences available in databases used for identification, further exacerbating the problem. To gain insights into the phylogenetic relationships within the complex, we performed whole genome sequencing on various strains from the BCCM/IHEM fungi collection. Phylogenetic analysis revealed a well resolved tree showing three clades representing the species T. mentagrophytes, T. indotinae and T. interdigitale. While all three species are genetically very close to eachother, the T. mentagrophytes genotypes

used in our analysis were closer to T. interdigitale than to T. indotinae, suggesting that T. indotinae might not be a clonal offshoot of T. mentagrophytes as has been suggested in previous studies. It also revealed that several publicly available genomes and even reference genomes are misidentified and thus are in urgent need of an nomenclatural update.

Cyanobacterial Taxonomy in the Age of Phylogenomics

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Taxonomic studies of cyanobacteria started in botanical literature in the early 18th century, and the traditional classification of cyanobacteria was based on morphological characters. The introduction of electron microscopy and phylogenetic analysis into taxonomic studies towards the end of the 20th century led to the development of a polyphasic approach, which considered the general morphology, ultrastructure, ecology and phylogeny of one to seven genes before assigning final taxonomic identity. Based on the polyphasic approach, numerous novel families, genera and species (including cryptic species) were described, and the taxonomic status of several families and genera was revised. Nevertheless, most of these descriptions and revisions were based on the 16S rRNA gene as the primary taxonomic marker. The limitations of this gene sequence in delineating the closely related species, intragenomic heterogeneity and the possibility of horizontal gene transfer have raised questions on the use of this marker gene in taxonomic studies. Nonetheless, the shortcomings of the 16S rRNA gene can be overcome by including a phylogenomic analysis (i.e., multiple-gene phylogenetic inference) in the polyphasic approach. However, the lack of consensus on the number of loci to be used, the unavailability of the reference material for some genera and inconsistent taxon sampling have hindered the development of a robust and consistent taxonomic framework for cyanobacteria to date. The rich diversity of axenic cyanobacterial strains available in the collection of Pasteur Cultures of Cyanobacteria (PCC) at the Institut Pasteur could be a valuable resource in developing a consistent taxonomic framework. A preliminary phylogenomic analysis using newly sequenced genomes of PCC strains (representing the phylum-wide diversity of cyanobacteria) together with the publicly available genomes indicates that several PCC strains belong to the novel, not-yet-described evolutionary lineages. Moreover, the phylogenomic analysis also demonstrates the need for the reassessment of certain families and orders as delineated according to the most recent classification system.

HAMBI/UHCC: A treasure trove of new cyanobacterial taxa and bioactive metabolites

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Cyanobacteria represent a cosmopolitan group of oxyphototrophic bacteria with unicellular, colonial and filamentous morphotypes. The immense diversity of cyanobacteria refers not only to their morphological variability and habitat preferences but also to a variety of functionally diverse and structurally complex bioactive metabolites they produce ranging from toxins to compounds with

potential pharmaceutical and/or biotechnological applications.
Founded in 1985 by prof. em. Kaarina Sivonen, the HAMBI/UHCC public collection is nowadays hosting over 1000 cyanobacterial strains most of which derived from Finnish freshwater habitats and the Baltic Sea followed by lichen-symbiotic cyanobacteria. Bioactivity screening of the abovementioned strains led to the discovery of many novel bioactive metabolites including, inter alia, anabaenolysins, muscoride B, deprenylmuscoride B, heinamides, nostoweipeptins, nostopeptolides, pseudoaeruginosins and varlaxins as well as many new variants of known natural products (e.g. microcystins, hassallidins).
Recently, a great number of strains isolated from terrestrial epilithic Finnish habitats as well as sciophilic habitats including the historically and culturally important Fortress of Suomenlinna (UNESCO) have been deposited to the culture collection. Ongoing polyphasic approach studies of the newly isolated strains indicate the existence of at least 2 new genetic lineages and at least 12 new species in Nostoc, Fulbrightiella, Roholtiella, Nodosilinea, Shackletoniella and Pseudanabaena amongst others. At the same time, results deriving from the morphological and phylogenetic analyses of previously isolated HAMBI/UHCC strains revealed the existence of several taxonomically interesting strains that deserve further study.
Altogether, this work highlights the importance of HAMBI/UHCC collection in expanding the biological and chemical diversity of cyanobacteria and the need for in depth study of the remaining HAMBI/UHCC cyanobacterial strains.

Data management, infrastructures

WDCM services as an information infrastructure for the exploration and utilization of microbial strains preserved worldwide

Keynote speaker: Juncai Ma
WDCM, China

The beauty of data and databasing

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Vincent Robert[1] and ChatGPT[2]
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Microbial data are essential for understanding and developing life sciences, as well as for providing solutions to various challenges in agriculture, environment, health and biotechnology. However, microbial data are often scattered, incomplete or inaccessible, limiting their potential use and impact. One way to improve the quality and availability of microbial data is to store them in culture collections, which are repositories of living microorganisms that preserve their genetic and phenotypic characteristics, as well as their associated data and metadata. Culture collections play a crucial role in providing authentic reference materials for scientists and industries, and in facilitating the exploration and utilization of microbial diversity. However, culture collections face many challenges, such as funding, sustainability, standardization, interoperability, and compliance with international regulations. Therefore, it is important to strengthen the coordination and collaboration among culture collections worldwide, and to integrate their data with other information resources, such as publications, patents, sequences and genomes, among many others. While the importance of assigning species names to strains is at the core of culture collection's activities, it should not be the only and ultimate goal of the latter. We all know that species names provide a common language for communication and identification of microorganisms, and, ideally, reflect their evolutionary relationships and biological properties. However, species names are not always consistent, accurate or even suitable for many applications. Therefore, it is necessary to develop systematic and standardized screening tools (phenotypic and genotypic) that would be automatically gathered, stored in efficient databases, connected automatically to data enriching third parties' data providers and analyzed by a range of diverse algorithms for multifaceted pattern discoveries. The use of artificial intelligence will be essential in this process, but the proper generation and storage of data is even more important.

The GEN-ERA toolbox: unified and reproducible workflows for research in microbial genomics

Luc Cornet
Luc Cornet, Benoit Durieu, Frederik Baert, Elizabet D'hooge, David Colignon, Loic Meunier, Valérian Lupo, Ilse Cleenwerck, Heide-Marie Daniel, Leen Rigouts, Damien Sirjacobs, Stéphane Declerck, Peter Vandamme, Annick Wilmotte, Denis Baurain, Pierre Becker

Microbial culture collections play a key role in taxonomy by studying the diversity of their strains and providing well-characterized biological material to the scientific community for fundamental and applied research. These microbial resource centers thus need to implement new standards in species delineation, including whole-genome sequencing and phylogenomics. In this context, the genomic needs of the Belgian Coordinated Collections of Microorganisms (BCCM) were studied, resulting in the GEN-ERA toolbox, a unified cluster of bioinformatic workflows dedicated to both bacteria and small eukaryotes (e.g., yeasts). This public toolbox is designed for researchers without a specific training in bioinformatics (launched by a single command line). Hence, it facilitates all steps from genome downloading and quality assessment, including genomic contamination estimation, to tree reconstruction. It also offers workflows for average nucleotide identity comparisons and metabolic modeling. All the workflows are based on Singularity containers and Nextflow to increase reproducibility. The GEN-ERA toolbox can be used to infer completely reproducible comparative genomic and metabolic analyses on prokaryotes and small eukaryotes. Although designed for routine bioinformatics of culture collections, it can also be used by all researchers interested in microbial taxonomy, as exemplified by our case study on Gloeobacterales (Cyanobacteria).

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Friday 22 September

Science policy, career skills, valorization of collections

How to write an influential review article

Keynote speaker: Daniela Ruffell
FEBS Letters, Germany

Writing a scientific Review article can be a daunting undertaking for the inexperienced author. Yet, drafting a Review provides a unique opportunity to learn all there is to know on your favourite topic, critically assess the body of knowledge, and finally present your own perspective to the scientific community. Through this lecture I aim to convey the full purpose of writing a Review, and provide useful advice to guide you through the process with guaranteed success.

Impacts of the exchange of strains between culture collections

Aurora Zuzuarregui
Spanish Type Culture Collection (CECT), University of Valencia

In 1982 the European Culture Collections' Organisation was borne to foster cooperation across political borders; to support science and to develop services for bio-and medical technologies; to serve as a platform to provide the necessary continuity and to form an incubator and umbrella for individual projects. Since then, ECCO collections have partnered in several joint projects and initiatives with a common ground; to facilitate access to microbial strains observing the legal framework and the European policies.
As part of the positive atmosphere of cooperation and trust, in the frame of ECCO and other initiatives or organizations (e.g. WFCC), collections have been exchanging strains in a one-to-one basis (equal balance). Since the publication of the first ECCO core MTA (Fritze, D. 2010), they do it under a verbal agreement with the premise that provider and recipient collection have equivalent MTA terms. In the meantime, the Nagoya Protocol has come into effect (2014), ECCO has published the second version of the ECCO core MTA (Verkley et al., 2020) and some ECCO collections participate in the recently launched European Infrastructure MIRRI-ERIC (2022). Besides, there is a growing concern about the environmental impact of any human action, including research activities. In this sense, the European Commission advocates for a climate neutrality by 2050, a great challenge that will require an evaluation of the activities of European organizations in terms of carbon footprint. All these, together with the fact that collections are very heterogeneous in terms of e.g. size, legal personality, funding sources or target users, while forced to operate on a sustainable and cost-effective basis, motivates opening a dialogue about the legal, environmental and financial implications of the exchange of strains between culture collections.
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Policy aspects related to EU Research Infrastructures

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Research Infrastructures (RI) are facilities that provide resources and services for research communities to conduct research and foster innovation of a unique nature that must be open to external users. They can be used beyond research, e.g. for education or public services, and they may be single-sited, distributed, or virtual. Microbial Resource RI (MIRRI) is a 'Landmark' in the Health & Food domain of the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap and is a distributed legal organization recognized as ERIC (European Research Infrastructure Consortium) by the Commission Implementing Decision (EU) 2022/1204 of 16 June 2022. With 26 ERICs set up since 2011, ERIC has become a legal instrument of choice for a large part of common European research infrastructure initiatives, notably for those prioritised by ESFRI Roadmap. This is helping to structure and integrate research activities and resources within the European Research Area (ERA). The ERA 2022-24 Policy Agenda lists several actions that strengthened the RI, creating added value and funding opportunities. The Pilar I (Excellent Science) of the Horizon Europa framework the RI have several opportunities to work in a collaborative way submitting projects to the different calls under the InfraServ (RI Services to support health research, accelerate the green and digital transformation, and advance frontier knowledge and InfraTech (next generation of scientific instrumentation, tools and methods and advanced digital solutions). All these opportunities will be presented and aligned with the MIRRI Strategic Research & Innovation Agenda 2021-2023.
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