# MICROBES Symposium by Young Researchers

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# Book of Abstracts

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# Session 1 - Health and Disease

# Loud Bacteria: How gas vesicles affect acoustic bacteria

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In recent years, advancements in synthetic biology, oncology, and microbiology have enabled the development of living biotherapies against cancer. Among them, bacteria naturally capable of penetrating and proliferating within the tumor microenvironment have been identified and engineered to produce various anticancer molecules. These therapeutic bacteria have proven effective against solid tumors in murine models. Despite these promising results, numerous challenges remain, especially in our capacity to monitor and control the distribution, colonization, and activity of these microorganisms once injected into the body. Tissues, impervious to light penetration, render classical fluorescence and luminescence techniques for tracking bacteria unusable in-depth within the organism.

Gas vesicles are intracellular gas-filled organelles naturally used by some cyanobacteria to adjust their buoyancy. Their mechanical properties also make them usable as contrast agents in acoustic imaging, detectable even deep inside tissues. Recent studies have demonstrated that the gas vesicle genes could be integrated into various synthetic genetic circuits and used as acoustic reporters in certain tumor-invasive bacteria to produce gas vesicles *in vivo*.

However, gas vesicles are voluminous structures requiring the expression of numerous genes. Their production dynamics and impact on bacteria could differ significantly from fluorescent proteins and must be understood to optimize their utilization.

Here we quantitatively study the dynamics of gas vesicles production, their impact on bacterial growth and metabolism, and their localization within cells. To achieve this, we employ various optical microscopy methods, electron microscopy, microfluidics, and acoustic imaging. Additionally, we are working on the development of *in vitro* assays using 3D tumor models. Our results indicate that gas vesicles tend to accumulate at one pole of the cell and affect cell senescence and growth rate.

## *Single-bacterium/Single-phage techniques to unveil novel virus-host dynamics during antibiotic stress*

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Phages, the viruses that parasitize and kill bacteria, were discovered almost a century ago by the clear and distinct plaques formed in the surface of bacterial lawns. In the following decades they were used as model for the study of fundamental biological processes as well as the development of powerful tools in molecular biology. Nowadays, phage study has regained interest, primarily due to the development of antibacterial therapies to cope with the antibiotic resistance crisis faced by medical systems worldwide. Additionally, following the discovery of CRISPR/Cas systems which serve as defence against the invasion by mobile genetic elements (MGE), there has been a surge in the discovery of many previously unknown and highly diverse anti-phage defence systems. Altogether, we are witnessing a revival in the study of phage biology with a highlight in phage applications and the ecological relevance of these viruses. The proper characterization of key parameters related to the phage replicative cycle is crucial for the development of adequate protocols in phage therapy and the study of phage-bacteria interaction within their ecological context.

Classical techniques to study of phage replicative cycles are directly linked to their propagation in populations of adequate bacterial hosts, and the collected data is generally an average of the individual infections taking place over the course of the experiment. While these techniques provide useful key parameters of phage propagation dynamics such as the adsorption rate, latent period, and burst size of a certain phage, they have as a caveat that they do not inform of the distribution and variability of phage-bacteria interactions. In this talk, innovative methods to track phage infection at single-cell/singe-phage resolution will be introduced. These techniques allow to determine the distribution of parameters such as adsorption, intracellular replication and latent period of individual infection events, by direct visualization of virus-host interaction in real time. Finally, we provide a method to directly observe the propagation of a viral epidemic in real time at microscopic level, with unprecedented detail. At each step we compare our results with theoretical and previous experimental data to confirm the validity of our measurements.

# Selective autophagy receptors in MHC-II-restricted antigen presentation and their highjacking by viruses to escape T cell immunity

***Gabriela Sarango***

CD4+ T cells play an important role in antiviral immunity. They are activated by virus-derived peptides presented by the major histocompatibility complex class-II (MHC-II) molecules in antigen presenting cells (APC). Autophagy contributes to the processing of cellular antigens and is often targeted by viruses. We first asked whether selective autophagy receptors (SARs) play a role in MHC-II viral antigen presentation. We show that TAX1BP1 strongly influences the loading and presentation of peptides by MHC-II molecules to CD4+T cells. We then used Human T-cell leukemia virus type 1 (HTLV-1) Tax protein, known to recruit TAX1BP1, OPTN and p62 to ask whether viruses target SARs to escape antiviral immunity. We show that the recruitment of OPTN by Tax is required to inhibit MHC-II-restricted viral antigen presentation and CD4+T cell activation. Altogether, we show that TAX1BP1 plays an important role in MHC-II antigen presentation and put forward OPTN as an HTLV-1 target to escape T cell immunity.

# Session 2 - Biotechnology and Engineering

# Process optimization of the baculovirus expression vector system for vaccine production

Jort Altenburg

The global population is rising, and an aging demographic, coupled with improved welfare, has led to an increase in age-related diseases, posing challenges to healthcare systems. Additionally, deforestation, climate change, and increased mobility have indirectly facilitated the emergence and spread of viral diseases, further straining healthcare resources. These factors have intensified the demand for vaccines and new medicines.

To address these challenges, the baculovirus expression vector system (BEVS) is a valuable tool. It enables the rapid production of complex recombinant proteins, to be used in the development of gene therapy and vaccine treatments. However, to meet the increasing demands for vaccines and gene therapy vectors, the BEVS must be optimized and improved to enhance large-scale biomanufacturing capabilities for clinical applications.

This project aimed to address issues associated with co-production of contaminating budded virus (BV) particles and to improve BEVS process yield. It pursued a dual purpose: first, to establish BV-free production using a temperature-sensitive baculovirus mutant, and second, to develop a scalable perfusion process to increase bioreactor production capacity.

## Improving microalgae biomass productivity by modulating photoprotection

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**ABSTRACT**

During cultivation in industrial photobioreactors (PBRs), microalgae can reach extremely high cell densities and therefore experience strongly variable light conditions due to mutual shading and mixing. This leads to the constant activation and inactivation of photoprotective mechanisms to shield the photosynthetic apparatus from light stress.[1] A main component of this photoprotection is the so-called xanthophyll cycle, in which the protective carotenoid zeaxanthin is produced from the light-harvesting carotenoid violaxanthin. However, the kinetics of the xanthophyll cycle and therefore of photoprotection, react slower than the experienced light fluctuation in PBRs, resulting in underprotected photosystems or energy loss.[2] By modulating the xanthophyll cycle in the industrially interesting microalga *Nannochloropsis*, we aimed to adjust the kinetics of photoprotection to the experienced light conditions, reducing photodamage and energy waste, thus enhancing photosynthesis. By accumulating the enzymes regulating the xanthophyll cycle, we could massively enhance the cycle kinetics, resulting in switch-like photoprotection corresponding to the time scale of light fluctuations in PBRs.[3] Performing growth analyses in flat-panel PBRs, we demonstrated that accelerating the xanthophyll cycle increased microalgae productivity by ca. 15% and could also improve the tolerance to high light stress. Taken together, we present that modulating photoprotection by altering the xanthophyll cycle can dramatically affect cell physiology of *Nannochloropsis* and improve their photosynthetic productivity depending on the light regime they are exposed to.

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# Role of bacterial membrane in cell elongation in *Bacillus subtilis*

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In bacteria, the cell wall (CW), an external layer mostly composed of peptidoglycan (PG), maintains cell shape and structural integrity. During cell elongation, the speed and location of CW insertion are mediated by two PG elongation machineries (PGEM) functioning semi-autonomously, namely the Rod complex and the class A Penicillin Binding Proteins (aPBPs) polymerases. Recently, it has been suggested that the plasma membrane (PM) may also play a role in this process. The PM is organised in different fluidity domains, based on the physical characteristics of the lipids and particularly the fatty acids that they contain. These domains have been linked to CW synthesis, with the Rod complex in particular localising to regions of increased fluidity.

During my PhD, I study how PM fluidity can influence CW elongation in the model Gram-positive bacterium *Bacillus subtilis*. First, I validated by gas chromatography that some exogenous fatty acids (eFAs) could be inserted efficiently in *B. subtilis* to change membrane composition. Using a Fluorescence Correlation Spectroscopy approach in live cells, I next demonstrated that the insertion of eFAs modifies membrane fluidity. Furthermore, I found that this leads to changes in cell width. In *B. subtilis*, this morphological feature is highly conserved during growth thanks to a balance between two complementary mechanisms: the thinning effect of the Rod system and the widening effect of aPBPs. By characterizing and quantifying the dynamics of MreB and PBP1, respectively, as proxies to these systems, I found that PBP1 seems to be affected by changes in membrane fluidity, whereas MreB is not. These results suggest that membrane fluidity may affect the activity of PBP1, pointing to a link between membrane organisation and CW synthesis.

Key words: *Bacillus subtilis*; cell wall elongation; plasma membrane fluidity; exogenous fatty acids; Fluorescence Correlation Spectroscopy

# Session 3 - Giving some space to microbes

# Exploring a new world: a dive-in into salt crystals

Lucas Bourmancé

High salt environments are ubiquitous in the solar system (Mars, Enceladus, Europa). On Mars, for example, these environments allow for the formation of evaporites. Those crystals represent a potential shelter for potential extant microorganisms as it can serve as a radiation shield.

As life on our planet is the only life we know of, this implies working on various extremophilic microorganisms to better understand and characterized how life can thrive in such conditions. Halophilic ("salt-loving") archaea including *Halobacterium* have been isolated as so-called "living fossils" preserved in the fluid inclusions of halite crystals (NaCl). Being able to recover archaea from halite crystals is of great exobiological interest as halite crystals have been identified on Mars. The model halophile *Halobacterium salinarum*, have previously shown resistance to several stress factors, including UV-C and gamma irradiation as well as desiccation, and offers numerous data allowing for detailed "-omics" analysis, thus being an ideal candidate for further analysis. However, much remains unclear concerning the molecular mechanisms under multi-stress conditions, such as combinations of solar irradiations, and the preservation value of evaporites under terrestrial and extra-terrestrial conditions.

This project has two goals. The first one aims at understanding how *Hbt. salinarum* responds to full-spectrum solar irradiation in liquid cultures and in halite crystals using ground-based solar simulators. Fluorescent markers added to liquid cultures will provide insights on physiological state evolution as well as impacts on their metabolic activity and structural stability. In parallel, TEM/SEM is being used to also provide new data regarding radiations effects on cell structure, with particular attention to the cell envelope as it has an exciting potential as biosignature.

The second axis focuses on the preservation of biomolecules as biosignatures of ancient life. Different natural fluid inclusion compositions are being tested to investigate the effect of different salts to preserve specific biomolecules from space radiations, mainly UV-C. Furthermore, the physical protection that offers the salt crystals is being evaluated.

Ground-based experiment will be compared to real space irradiation as samples will be sent on the space exposure platform Exocube, which will be part of the ExoBio platform on the ISS. For the first time, a biological exposure platform will allow for the observation of organisms' responses to the space environment in real time, using fluorescent markers and *in-situ* monitoring technology. Additional post-flight analyses of cellular structures will provide further insights into the preservation of microorganisms within halite brine inclusions.

Thus, this project is aiming at determining, on a molecular level, how *Hbt. salinarum* adapts to space-induced multi-stress irradiation, using a whole new *in-situ* monitoring system on the ISS as well as ground-based experiments. This will allow for a better understanding of how potential halophilic life could thrive and be preserved inside evaporites on other planets. In addition, post flight cell structure analysis will provide information to help characterize of new biosignatures.

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# Acoustic Levitation for Life Support System (ALLISS)

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Cyanobacteria are photosynthetic microorganisms that utilize carbon dioxide to produce oxygen. Consequently, these organisms are a feasible option for space applications, particularly in the context of biological life support systems (BLSS) for long-term space missions. Space agencies, such as the European Space Agency, are actively engaged in developing life support system programs, exemplified by the Micro-Ecological Life Support System Alternative (MELiSSA) project. However, enhancing the efficiency of photosynthesis is imperative to expedite the conversion of carbon dioxide into oxygen. An essential factor is the level of microbial exposure to light.

Bioreactors are commonly used to study the growth and function of microorganisms. Typically, these devices have high capacity and are used to monitor changes in carbon dioxide and oxygen levels. Throughout the experiment, the culture's pH and temperature are also constantly monitored. This data can be used to evaluate the development of cyanobacteria under various settings.

Here, we will describe a novel method for cultivating cyanobacteria in a bioreactor. We have designed a technique that enables us to levitate cyanobacteria in bulk and arrange them in sheets. With this technique, we anticipate that more light will penetrate the bulk of the liquid, enhancing photosynthesis. Currently, it is unclear what the most critical conditions for levitating cyanobacteria are, or whether levitating the cells has any impact on them. Thus, research is now underway to determine whether levitation may increase the efficiency of bioreactors.

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# Short-term memory effects in the phototactic behavior of microalgae

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Phototaxis, the directed motion in response to a light stimulus, is crucial for motile microorganisms that rely on photosynthesis, such as the unicellular microalga *Chlamydomonas reinhardtii*. It is well known that microalgae adapt to ambient light stimuli. When stimulating *C. reinhardtii* on time scales of several dozen minutes, the response of the microalga evolves as if the light intensity were decreasing [1].
In our work, we show that microalgae also have a short-term memory, on the time scale of a couple of minutes, which is the opposite of an adaptative behavior. At these short time scales, when stimulated consecutively, the response of *C. reinhardtii* evolves as if the light intensity were increasing.
Our experimental results are rationalized by the introduction of a simplified model of phototaxis. Time-integration of light stimuli, or memory, results from the interplay between an internal biochemical time scale and the time scale of the stimulus; as such, these memory effects are likely to be widespread in phototactic microorganisms.

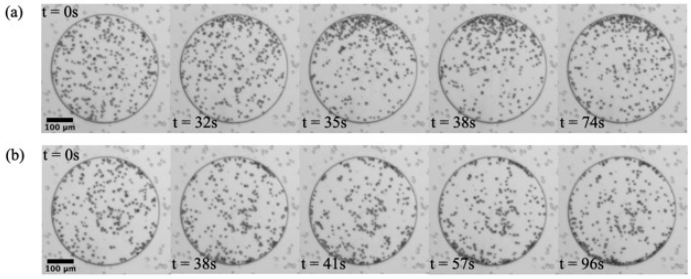


**Fig. 1:** *C. reinhardtii enclosed in a well are exposed to blue light stimulus coming from the upper side of the wells. Two consecutive experiments are performed with 1min30s rest in between. The algae show positive phototaxis in the first experiment (a) and negative phototaxis in the second experiment (b).*

## References

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# Session 4 - Ecology and Evolution

## Reduction and Biosorption of Silver Nanoparticles by Bacteria

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Successful use of bacteria in heavy metal remediation depends on their resistance to toxic metals and their ability to reduce and trap metal ions. The current study provides, using SEM, TEM and AFM, new insight into the ability of *R. gelatinosus* (and *E. coli*) cells to reduce silver ($Ag^+$) and trap silver nanoparticles (Ag-NPs) at their surface. According to EDX spectra analysis of whole cells, the Ag-NPs correspond mostly to silver chloride. Size distribution suggested several, may be competitive, stochastic nucleation processes. Together with the efflux system, this biosorption activity should function as part of the survival strategy allowing cells to withstand metal poisoning.

Like studies showing differential antibiotic susceptibility and the presence of subpopulations within planktonic or bacterial biofilms, we found that only a small subpopulation of cells had the ability of Ag+ removal through the biosorption of Ag-NPs and act as scavengers and first defense line. We hypothesized that this striking phenotype was very likely related to metabolic heterogeneity introduced by variability in the physiological state of cells within the population. Future breakthroughs in bioremediation efforts will rely on the ability to separate subpopulations and the full characterization of the molecular components or factors involved in NPs biosorption.

Identifying those factors could be exploited to target metal nanomaterials to specific bacterial cells. On one hand, we disposed of probes for phenotypic heterogeneity, and on the other hand, hacking this defense mechanism can also allow nanoparticle bio-design using bacteria.

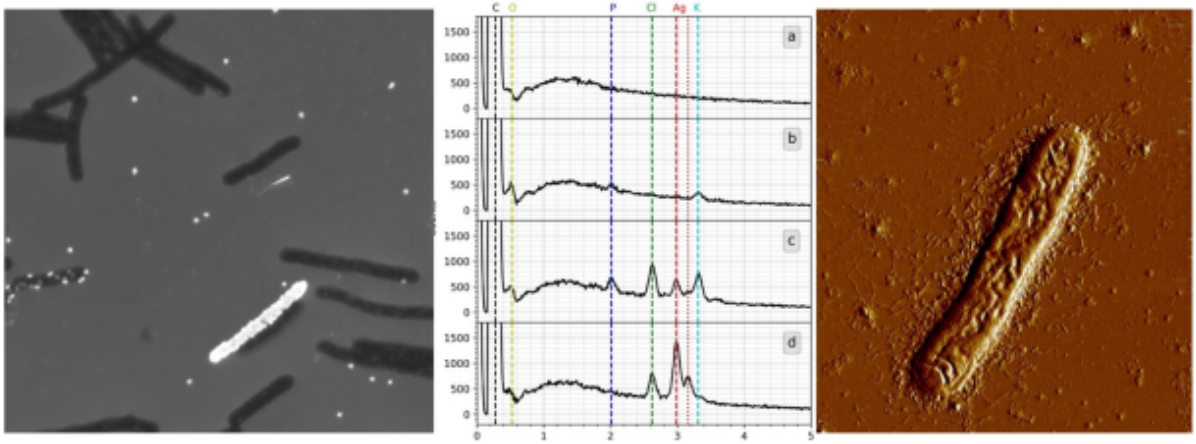


Figure 1: SEM, AFM images and EDS spectra of silver nanoparticles and bacteria interaction

## Continuous culture in custom computer-controlled mini-bioreactors

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Molecular microbiologists seldom use bioreactors despite the interest of these continuous culture systems. In order to facilitate their implementation, we developed a new modular system of computer-controlled mini-bioreactors which takes advantage of the opportunities offered by small-scale digital fabrication (Fablabs) and the spread of powerful programmable and connected microcontrollers that led to the Internet of Things (IoT).

The combination of modularity and small culture volumes makes it possible to implement complex experimental designs involving real-time monitoring and coordinated control of several bioreactors. Envisioned applications range from experimental and directed evolution to study of physiological adaptation. As a proof of concept, we applied our modular system to maintain *Bacillus subtilis* cultures in various conditions: from simple parallel chemostats to a cascade of two chemostats that maintains a continuous culture of phages. Genomic data on the evolution of resistance to increasing ethanol stress will be presented.

# Strain-level variation alters host-symbiont interaction dynamics in the Halorubrum lacusprofundi – Candidatus Nanohaloarchaeum antarcticus system

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*Candidatus* Nanohaloarchaeum antarcticus is a DPANN archaeon that is an obligate symbiont of *Halorubrum lacusprofundi* and requires direct cell-cell interactions with its host for survival[1]. As part of these interactions *Ca.* Nha. antarcticus takes up nutrients directly from its host including lipids which it lacks the enzymes necessary to synthesis itself. Previous studies of other DPANN archaea have concluded there is no difference in lipid composition between DPANN symbionts and their hosts, but it is not clear how universal this is for DPANN – host systems. To investigate the lipid recruitment of *Ca.* Nha. antarcticus from its host we applied an untargeted lipidomics approach to purified nanohaloarchaeal cells as well as a time series of co-cultures and pure *Hrr. lacusprofundi* cultures. Our results show both qualitative and quantitative differences between lipid profiles of *Ca.* Nha. antarcticus and *Hrr. lacusprofundi* indicating the nanohaloarchaeon is selective in recruiting lipids from the host. Additionally, co-culture biomass displayed significant differences in lipid composition compared to pure *Hrr. lacusprofundi* cultures reflecting a shift in the host lipidome as a consequence of interactions with the nanohaloarchaeon. These differences are likely to impact membrane structure and fluidity as well as efficiency of energy production. In environmental communities it is likely that the shifts in lipid profile impact host capacity to adapt to stressors.

Keywords: Nanohaloarchaeota, Symbioses, Genomics

References: Left aligned, Arial 9.

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# Session 5 - Agriculture and Food

# Characterisation of a biofilm at the air-liquid interface: Toward a link between biochemical composition and physical properties

Emmanuelle Baudu

Bacillus amyloliquefaciens is a soil bacterium known for its role in promoting plant growth. In soil, the presence of bacteria producing extracellular polymeric substances (EPS) can have an impact on the stability of soil microaggregates. Therefore, understanding the links between biochemical composition of a biofilm and its physical characteristics is critical for agronomy research.

Under lab experimental conditions, *B. amyloliquefaciens* can produce a resistant biofilm at the air-liquid interface. Pellicles of *B. amyloliquefaciens* L17 strain were imaged (AFM, SEM) to describe cell morphology and EPS on each side of the pellicle. Then the EPS composition of pellicles was explored by: (i) a sequential extraction protocol with quantitative analysis of extracellular proteins, olysaccharides, and DNA; (ii) attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) on each side of dried pellicles, in order to differentiate EPS composition at the two interfaces. Finally physical properties were analysed: (i) mechanical resistance; (ii) water retention capacity by desiccation.

Starting from identical inoculum, pellicles of L17 showed different phenotypes, L17a and L17b, for which the physicochemical behaviour of the pellicle variated considerably. Electronic microscopy revealed different cell morphologies and EPS productions between L17a and L17b and between pellicle interfaces. These results were reinforced by the desiccation curves, clearly underlying a higher water holding capacity in L17a, which could be explained by the presence of different type of EPS in the pellicle. Among the EPS extracted and quantified, DNA was detected in higher quantity in L17a extracts than in L17b. All these results demonstrate that a single strain is able to produce very distinct biofilms regarding cohesiveness, water retention and composition.

Characterization of the extracellular polymers responsible for these physical variances is under investigation. Establishing the links between EPS composition and physical characteristics of the biofilm will allow a better understanding of biological mechanisms involved in soil aggregation and will be beneficial for agronomy in the long run.

Keywords : Bacillus amyloliquefaciens, EPS, biofilm

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## Bacteriophages: can we predict their impact on cheese-making?

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Milk acidification is a key step in the cheese-making process. In the industry, bacteriophages can attack lactic acid bacteria (LAB), which are responsible for the conversion of lactose to lactic acid. In consequence, acidification can be reduced or even stopped, leading to a halt in the production. This results in the waste of raw materials as milk is discarded, severe economic losses for cheese-makers, and negative impacts on the environment. The goal of this study is to develop an unstructured dynamic model to predict the dynamics of phage attack.

To build the model, acidification curves were generated for different couples of initial LAB concentrations and phage titers. A dynamic mechanistic model was then constructed and consisted of 5 ordinary differential equations for the state variables: lactose and lactic acid concentration, susceptible and infected LAB concentration, and phage titer. The model parameters were estimated from the observed data. The model was analyzed and new optimal experiments were designed with different initial conditions and additional measurements. The model could successfully predict the success or failure of milk acidification. Important biological parameters were deduced from simple, low-cost acidification measurements. These parameters included bacteria's maximum growth and lysis rates, phages' burst size, etc. Sensitivity analysis helped identify biologically relevant aspects of phage-host interactions. This knowledge can be used to develop easy routine strategies to fight phage attack in the dairy industry. The model can be used to raise awareness amongst cheese makers on the importance of cleaning to avoid food and material waste.

**Phage bacteria co-evolution in wine. The virulent Krappator 27 phage selects for unique genomic changes in the lactic acid bacterium *Oenococcus oeni.***

Florencia Oviedo-Hernandez, Amel Chaïb, Olivier Claisse and Claire Le Marrec

The lactic acid bacteria *Oenococcus oeni* is an essential player in the winemaking process. Previously, oenophages have been isolated and characterized suggesting that phages are an unexplored actor in the wine environment. In this study, we investigated how the bacterial genome evolves during phage infection. Co-evolution assays were carried out in MRS Broth in four replicates. The experimental design included a total of 19 transfers (around 95 generations) for each evolved and coevolved culture. Bacterial and phage populations were enumerated at the early steps of the experiments (transfers 1 to 5) and at the end (transfer 16 to 19). The phage population decreased significantly at the final transfers in 3 of 4 replicates and extinction was observed in one culture. Individual colonies were isolated at relevant steps (T3, T16 and T19) and their resistance towards Krappator 27 was assessed. The spectra were extended to the virulent phage Vinitor162 and the ex-temperate phage OE33PA[1,2,3], which are expected to use different receptors in *O. oeni*. Clones with distinct panel of resistance/sensitivity were selected over time. At early transfer a diversity of resistant phenotypes were found, while at the later transfers only one type of resistance was found, where clones were resistant to the original phage Krappator 27 and OE33PA but completely sensitive to Vinitor 162, suggesting that selection of more fitted clones took an import role at the end of the experience. Representative colonies were further selected and their genomes sequenced, alongside evolved and coevolved phages. Through BreSeq analyses, we observed an array of mutations in cell wall macromolecule- encoding genes, and their analyses will give clues to identify the receptor on the surface of the host cell.

1, Chaïb et al., 2022 Int J Food Microbiol. 2022;383:109936 ; 2, Chaïb et al., 2019, Appl Environ Microbiol. 85(19):e00997-19; 3, Philippe et al., 2021, Front Microbiol. 2021;11:596541.

# Posters

**Deciphering the role of the oocydin in the colonization of Solanum tuberosum by the phytopathogen Dickeya solani in confrontation with the root micro-biota**
Amaro-Lauer Coline

In emergence in Europe, *Dickeya solani* is a necrotrophic bacterium targeting *Solanum tuberosum*, the potato plant, representing millions of euros of loss per year for the potato plant sector. Recent genomic studies have highlighted the *ooc* cluster as important for root colonization of the plant host. This cluster encodes for a specialized metabolite with an antimicrobial activity, the oocydin. This study aims to better understand the impact of the production of oocydin in host colonization in confrontation with the resident root microbiota using a comparative approach between wild type and mutant strains for chemical, *in vitro* and *in planta* analysis.

## Single-filament imaging of the actin homologue MreB

Ingrid Adriaans

MreB, a bacterial protein homologous to eukaryotic actin, is prevalent in most rod-shaped bacteria. *In vivo*, MreB assembles into filamentous structures along the cell membrane, serving as a scaffold for the cell wall biosynthetic machinery and exhibiting circumferential motion around the cell periphery.
How MreB assemblies are formed and what their precise ultrastructure is *in vivo* remain unknown. Our lab has recently overcome the main bottleneck for the study of Gram-positive (G+) MreB *in vitro* and we are now able to purify functional MreB from *Bacillus subtilis* and fluorescently label it.
To investigate how MreB filaments are formed on a lipid surface and characterize their behavior, we use single filament Total Internal Reflection Fluorescence (TIRF) imaging in an *in vitro* reconstitution assay on a Supported Lipid Bilayer (SLB) that mimics the bacterial membrane.
We show polymerization of MreB filaments on the lipid bilayer that quickly form a dense network of filaments. Strikingly, MreB filaments can deform the membrane and induce large membrane protrusions that grow out of the lipid bilayer.
These findings contribute valuable insights into the fundamental mechanisms governing MreB assembly and offer new perspectives on the dynamic interplay between MreB and the cell membrane.

**Rhizosphere microbiota evolution under water deficit conditions improves growth of *Brassica juncea***

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## Short sentence

The evolutionary conditions of the rhizosphere microbiota influence its impact on the plant.

## Abstract

Rhizosphere microbiota manipulation emerges as a promising practice to enhance plant tolerance to drought. In controlled greenhouse conditions, we used experimental evolution on the rhizosphere microbiota under water deficit conditions to evaluate its impact on the growth of *Brassica juncea*. During 10 successive growth generations of *Brassica juncea*, we randomly selected rhizosphere microbiota under either moderate water deficit (pF=2.3) or extreme deficit (pF=3.5) and transferred them to the next plant generation. We investigated the evolution of the rhizosphere bacterial and fungal community structure by amplicon sequencing, and changes in plant phenotype (projected leaf area, height, width…). Both bacterial and fungal communities' evolution across the 10 generations were influenced by the intensity of water deficit. This influence could be explained by the selection pressure exerted by host plants, particularly towards bacteria. The improvement of the microbiota's impact on plants dur to experimental evolution resulted in an increase in leaf surface area by 7.6% and 27.3%, in the moderate and extreme water deficit respectively. These results confirm the real the potential of direct rhizosphere microbiota manipulation and selection for enhancing the host plant phenotype under water deficit conditions.

**Investigating deep-sea vents microbial ecosystems using microfluidic approaches**

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Deep in the Earth oceans, deep-sea vents (DSVs) offer unique ecosystems that support life, without photosynthesis, on a wide range of steep physical and chemical gradients. DSVs are hypothesized to represent promising environments for the emergence and proliferation of life on Earth during the Hadean, and within the Ocean Worlds in our Solar system (such as Europa, Titan, and Enceladus). But these ecosystems are still poorly understood, and a lot of questions remain on i) the DSVs microbial population and lifestyle, ii) the limits of life in these environments and iii) the impacts of extreme conditions on both habitability and life detection in these systems. In the frame of my PhD project, I study these specific environments, and representatives of archaea living within them, at lab scale using microfluidics under extreme conditions. These microreactors reproduce the dynamic geochemical properties, as well as the porous confined environments of DSVs. The objectives of my PhD are: (i) to perform fast-screening phenotyping of DSVs microbial life to determine their adaptation strategies and their boundaries (while coping with high pressure, heavy metals concentrations and thermo-chemical gradients conditions) and (ii) to investigate the DSVs chimney minerals - microbial interactions, and colonization. The ultimate goal is to decipher both the microbial diversity, their dynamics within the DSVs, and their resilience strategies, in order to bring new input about DSV ecosystems biosignatures (*i.e.* biomolecules, metabolic function, cellular morphologies and biomineralization).

## Direct measurement of membrane fluidity in *bacteria* using TIR-FCS

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Bacterial cells need to constantly maintain the fluidity of their cytoplasmic membrane in response to external stimuli such as changes in temperature, growth medium, etc. Bacterial membrane fluidity is therefore a crucial element of phenotype which was so far mostly assessed indirectly, via analytical methods such as fatty acid analysis or using environment-sensitive dyes in live bacteria. These dyes, like Laurdan, insert in the cytoplasmic membrane and change their fluorescence emission properties when the local environment changes, indirectly informing about local membrane fluidity variations. The information provided by these methods is however qualitative and can lead to ambiguous results. Here, we introduce a method to quantify membrane fluidity in live bacteria by measuring the diffusion speed of the membrane marker Nile Red using fluorescence correlation spectroscopy (FCS), a gold-standard technique in membrane dynamics investigations in eukaryotic systems, on a total internal reflection fluorescence microscope (TIR-FCS). To validate this assay, we characterised a well-studied phenomenon: the recovery of membrane fluidity upon cold shock (transfer from 37°C to 20°C) in the model Gram-positive rod-shaped bacterium *B. subtilis*. Previous experiments revealed that upon cold shock, the membrane fatty acids of *B. subtilis* are remodeled to compensate for the cold-induced loss of membrane fluidity. With TIR-FCS, we observed the expected recovery in membrane fluidity, while we also found that this compensation is not complete, as membrane fluidity is lower at 20°C than at 37°C. Finally, we performed the same experiments in the Gram-positive spherically-shaped pathogen *Staphylococcus aureus*, in which cold shock response has been scarcely studied. Our results provide exciting new insights into the dynamics of the plasma membrane in Gram-positive bacteria and our methodology based on TIR-FCS has a significant potential to investigate a wide range of molecular dynamics and physiological adaptations in bacterial cells.

## DNA topology in Archaea: an uncharted land

Basta-Le Berre Tamara

Chromosomal DNA supercoiling (DNA topology) is now recognized as an important epigenetic marker for the control of gene expression but also for DNA replication and repair. Deregulation of DNA supercoiling homeostasis occurs in cancers, neurodegenerative diseases and some autoimmune diseases, however, the underlying molecular mechanisms are still poorly understood. Archaea are prokaryotic microbes that are now considered to be the ancestors of all eukaryotes. These microbes are particularly interesting models to study DNA topology, as most of them have histones and DNA replication and transcription mechanisms homologous to those of Eukaryotes, but in a less complex cellular context and therefore easier to dissect. DNA topology is regulated in all cells by dedicated enzymes called DNA topoisomerases. The physiological role of these enzymes is poorly understood especially in Archaea where the necessary tools are yet to be developed. We are currently developing such tools[1,2] and our ultimate goal is to understand how DNA supercoiling homeostasis is achieved in Archaea through the action of DNA topoisomerases and how DNA topology contributes to the overall 3D architecture of archaeal chromosomes.

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## High-Throughput Image-Based Cell Screening to Identify Compounds to Improve Tuberculosis Treatment

Blanca Bernabeu Vilaplana[1], Jeanne Chiaravalli[2], Cécile Tillier[1], Anne Danckaert[3], Fabrice Agou[2], Nathalie Aulner[3], Roland Brosch[1], Ludovic Tailleux[1]

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### Abstract
Tuberculosis (TB), which is caused by *Mycobacterium tuberculosis* (MTB), is the deadliest disease due to a single infectious agent, ahead COVID-19, HIV/AIDS and malaria. An effective vaccine against TB is still not available and multidrug resistant (MDR) strains of MTB are continually emerging. Drug-sensitive TB can be treated with a 6-month course of up to 4 antibiotics. However, the ability to cure MDR-TB is more difficult, requiring longer treatments with more toxic and costly drugs. New strategies are thus urgently needed to prevent the emergence of drug resistance, to shorten treatment duration, and to limit treatment-associated side effects. In addition to the development of classical drugs targeting key factors in MTB physiology, host-directed approaches have emerged as a promising strategy to be used in adjunct with existing or future antibiotics.
Here, we present a high-content, image-based method for identifying host-targeted molecules that may improve the efficacy of anti-TB drugs. Human macrophages were infected with GFP expressing MTB and treated in 384-well plates with antibiotics in conjunction with an FDA-approved drug library. Intracellular bacterial growth and cell viability numbers were evaluated using the Opera Phenix Plus high-content screening system. Preliminary results have demonstrated the efficacy of our approach to identify molecules that improve the efficacy of two anti-TB drugs, namely bedaquiline and linezolid.

# Controlled transport of passive beads by phototactic swimming micro-organisms

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Passive particles immersed in an active bath of micro-swimmers, either artificial swimmers or living microorganisms, may be displaced due to the activity of the suspension [1]. This enhanced motion can lead to rich phenomena such as aggregation or phase separation [2]. In our experimental work, we study how passive beads are moved by directionally swimming micro-organisms. Considering their quick reaction to light [3], we use micro-algae *Chlamydomonas reinhardtii* as our biological micro-swimmer.

In a closed oxygenated chamber, we add passive beads of varying diameter $D_B$ and density to a dense suspension of *C. reinhardtii*. As their concentration locally grows due to the incoming light, the algae destabilize and create convection cells around them. Centered on the highest local algal concentration, these vortices generate an outward flow on the bottom side of the chamber, and an inward flow on the topside. The beads denser than the medium are thus pushed away, while the lighter beads are attracted to the high algal concentration. By varying the incoming light direction, we manage to create complex patterns of passive particles, and even direct them towards precise locations. Such directed motions of micro-particles open up exciting perspectives, for instance in medicine.

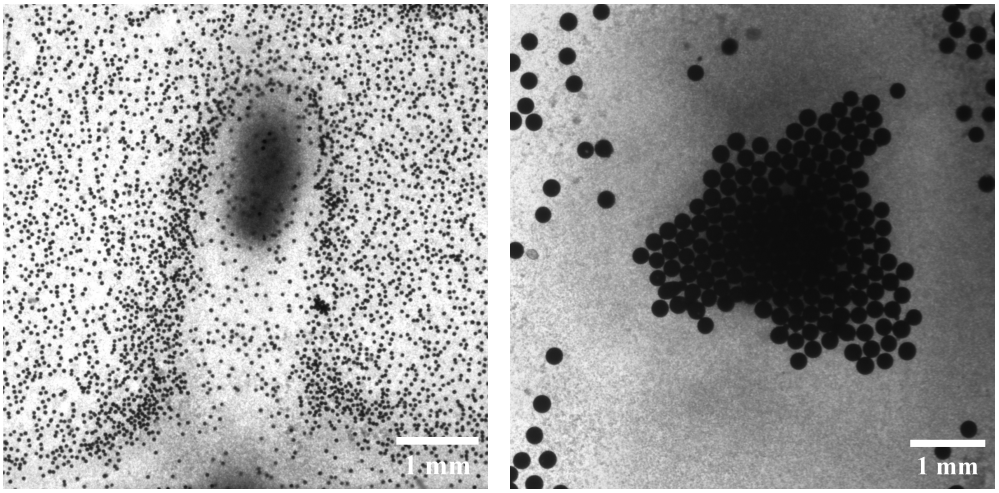


**Fig. 1:** *Bright field images of passive beads in a suspension of micro-algae directed by light. High concentrations of algae (darker background) create local convection cells. (a) Located on the bottom surface, heavy beads of diameter $D_B = 50\ \mu m$ are repelled by the convection cells. (b) Located on the top surface, light beads of $D_B = 230\ \mu m$ are attracted by the convection cells.*

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# Imputation of missing antibiotic susceptibility values in *Pseudomonas aeruginosa* using machine learning

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**Background**
*Pseudomonas aeruginosa* is a challenging pathogen with complex antibiotic resistance patterns. Minimum Inhibitory Concentration (MIC) testing strategies in clinical settings often involve a limited number of antibiotics, which does not provide a global overview of the bacterial strain phenotype.

**Methods**
In this study, we develop machine learning-based models to impute missing MICs based on known MICs from other antibiotics. We use the dataset from Stanton et al. [1], containing MICs values for 1019 *P. aeruginosa* strains and 15 antibiotics.

A first analysis to understand the correlation between the MICs of the different antibiotics generally shows a strong correlation between the MICs of antibiotics within the same family (beta-lactams, fluoroquinolones, aminoglycosides), except for colistin. Therefore, we generated a new dataset without colistin, by simulating missingness in the original dataset with a Missing At Random (MAR) strategy with the condition to have at least one non-missing value by family.

This generated dataset is used to train several imputation methods, including a univariate imputer, a multiple imputation by chain equations (MICE), and a denoising autoencoder.

**Results**
The best performances was majoritary obtained with the denoising autoencoder, with a 1-tier accuracy from 80% to 98% on the missing MICs depending on the antibiotics. Moreover, the accuracy varies depending on the number of missing MICs.

**Conclusions**
Our work provides a machine learning-based model for imputing missing MICs values in *P. aeruginosa*. This tool offers interessting antibiotic susceptibility predictions within specific dilution ranges for selected antibiotics. We believe it will be useful in clinical settings to get a complete picture of the antibiotic resistance profile for a specific strain using a limited amount of information.

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*Beyond bacterial cell lysis: antibiotic impact on Pseudomonas aeruginosa biofilm streamer formation*

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INTRODUCTION AND AIMS: Nosocomial infections associated with medical devices are a significant global health issue, often linked to biofilm formation. There's growing evidence suggesting that biofilm streamers—suspended, thread-like biofilms—play a critical role in initiating and perpetuating these infections. Our research aims to elucidate the impact of streamers in biofilm-related infections on biomedical implants.

METHODOLOGY: To investigate Pseudomonas aeruginosa biofilm streamer formation, we integrate microfluidics with microscopy and image analysis. Our approach utilizes a cutting-edge microfluidic platform equipped with isolated micropillars to induce the growth of suspended, filamentous biofilms, known as streamers. These structures, observed in diverse clinical and environmental scenarios, are particularly intriguing due to their potential influence on biofilm-associated infections.

RESULTS: Our study identifies extracellular DNA (eDNA) as a crucial structural element in Pseudomonas aeruginosa biofilm streamers. Utilizing COMSOL Multiphysics for simulation, we've developed a model to dissect the intricate dynamics of these streamers. Our data underscores the strain-specific formation of biofilm streamers in P. aeruginosa. Additional experimentation reveals that DNase effectively inhibits streamer formation, highlighting the fundamental role of eDNA, while antibiotics, notably ciprofloxacin, seem to enhance their density.

CONCLUSIONS: Biofilm streamer formation is distinctly strain-dependent, underscoring the unique characteristics of different Pseudomonas strains. Antibiotics, particularly ciprofloxacin, are shown to modify the morphology of biofilm streamers. This research paves the way for strategies to optimize implant materials, diminish biofilm formation, and ultimately improve clinical outcomes for patients with implant-based treatments.

**Minibioreactor arrays (MBRAs) to model microbiome response to tryptophan and alcohol in the context of alcohol-associated liver disease (ALD)**

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**Background and Aims:** Intestinal microbiota (IM) plays a causal role in the severity of alcohol-associated liver disease (ALD). Using IM transplantation in mice, we proved that the dysbiosis of alcohol use disorder (AUD) patients with severe alcohol-associated hepatitis (sAH) could be modified, leading to an improvement in alcohol-induced liver injury by increasing tryptophan metabolites to activate aryl hydrocarbon receptor (AhR) signaling pathway. However, the effect of tryptophan on IM in AUD patients, as well as its interactions with alcohol, remain to be elucidated. For this purpose, we used an in vitro approach with Minibioreactor arrays (MBRAs) that allows for the study of IM in a continuous-flow culture with well-controlled factors.

**Method:** Fecal samples from AUD patients with sAH (n=2) or with noAH (n=2) were transferred to MBRAs chambers. After 24 hours of adaptation in the initial medium, treatments with different tryptophan concentrations (low: 8mg/L, normal: 24mg/L and high: 72mg/L) were initiated for 48 hours. Subsequently, alcohol was introduced in the system for 5 days (50mM ethanol/Day). Finally, alcohol was removed and the cultures were maintained for an additional 5 days. IM analysis was conducted by 16s sequencing. AhR activity of tryptophan derivatives in supernatants was determined using two reporter lines: intestinal epithelial cells (HT-29) and hepatocytes (HepG2) labelled with Lucia-AhR.

**Results:** After 24h of stabilization, MBRA effectively maintains each fecal community. Tryptophan had no effect on the alpha and beta diversity of the IM from sAH and noAH patients. However, normal tryptophan level decreased the relative abundances of *Escherichia – Shigella* and increased *Bacteroides* in noAH IM, decreased *Proteobacteria* and increased *Bacillus* in sAH IM. In the absence of alcohol, tryptophan changed more number of bacteria in noAH IM (43 species) than in sAH IM (8 species). However, with alcohol

conditions, tryptophan had minimal effect on the noAH IM. Compared to low tryptophan, normal and high tryptophan levels increased the AhR activity.

**Conclusion:** Our results suggest that maintaining a normal tryptophan level in patients with noAH could be essential to prevent dysbiosis and high concentrations of tryptophan may have a beneficial effect on the IM of sAH patients. Tryptophan holds potential as a novel therapeutic agent for ALD treatment but these results must be confirmed in vivo.

# Exploring chromatin dynamics in *Streptomyces*, antibiotic-producing bacteria

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*Streptomyces* are gram-positive, multicellular, filamentous aerobic bacteria characterized by a large linear chromosome divided into a central region harboring cores genes and two extremities enriched in genomic islands (GIs) such as the specialized metabolite biosynthetic gene clusters (SMBGCs) and a prophage. Nevertheless, the majority of GIs remain transcriptionally silent over growth under lab conditions. Interestingly, a stress condition (HT medium) was found recently to be associated with awakening of a prophage. Characterizing *Streptomyces* physiology and chromosome conformation in this growth condition and identifying the cellular machineries involved in the regulation of prophage expression are hot topics to explore. First, we have identified a variant of HT medium named BM (Bacteriophage production Medium) in which the phage production is optimal and we demonstrated that the production of this phage mediates multicellular bacteria dispersal in response to metabolic stress. Moreover, we studied the effect of the prophage on chromosome conformation and results have shown that phage dormancy is associated with a specific DNA contact pattern, named condensate, and this motif has disappeared when the phage is produced. In addition, by performing the Chromatin ImmunoPrecipitation followed by sequencing (ChIP-seq), we found out that Lsr2A, which is a nucleoid associated protein (NAP) that acts as xenogeneic silencer, targets the prophage and this reflects the involvement of this NAP in the prophage modulation. The project is ongoing and more experiments will be done to better explore the effect of prophage on chromosome conformation and gene expression as well as the role of other NAPs in the chromatin dynamics of *Streptomyces* over growth.

## ***Ruminococcus gnavus* in Spondyloarthritis, story of a commensal killer**

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**Background**: Spondylarthritis (SpA) is a group of chronic inflammatory disorders characterized by osteoarticular and extra-articular manifestations, including inflammatory bowel disease (IBD). Predisposition to SpA is determined both by environmental and genetic factors, among which the class I major histocompatibility complex (MHC) allele HLA-B27 is the strongest. Although described nearly 50 years ago, the mechanism behind such striking association remains unsolved. To date, SpA pathogenesis remains largely unexplained. Growing evidence have highlighted a potential role of gut dysbiosis in SpA. We recently found that the abundance of *Ruminococcus gnavus*, a gram-positive strictly anaerobic bacterium naturally present in the gut of healthy controls (HC), is increased during SpA in correlation with disease activity.

**Aims**: In this study, our goal is to determine if some *R. gnavus* strains are specifically associated with SpA and if they are pathogenic.

**Methods**: *R. gnavus* colonies were isolated from colonic biopsies and stools of SpA patients and HCs. Validation of *R. gnavus* species was determined by specific qPCR before sequencing. Immunogenicity of *R. gnavus* strains was evaluated by their ability to induce mortality and proinflammatory cytokines secretion after culture with peripheral blood monocytes isolated from SpA patients.

**Results**: We successfully isolated *R. gnavus* strains from 5 HCs and 16 SpA patients. Among them, 18 different strains have been identified by sequencing and do not overlap between SpA patients and HCs. Importantly, *R. gnavus* strains isolated from SpA patients have different levels of cell toxicity and proinflammatory functions on monocytes than the one isolated from HCs.

**Conclusions**: Our work demonstrates that *R. gnavus* diversity is vast and highlights a strain-specific pathogenicity. Further studies are required to better understand the type of cell death induced by *R. gnavus* and its role during SpA.

**Lipid droplets in hepatocytes support *Enterococcus faecalis* intracellular multiplication**

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Abstract:

*Enterococcus faecalis* is an opportunistic Gram-positive pathogen responsible for hospital- and community-acquired infections. To date, *E. faecalis* is also one of the rare bacteria for which a link between intestinal overgrowth and the severity of alcohol-related liver damage has been demonstrated. Severity and mortality of alcoholic hepatitis are consistent with the presence of *E. faecalis* expressing cytolysin, a toxin capable of lysing bacteria and cells. In the mouse liver, *E. faecalis* are recognised by the resident macrophages, leading to inflammation and hepatic lesions. We have recently shown that *E. faecalis* multiplies and survives in hepatocytes. This intracellular lifestyle is associated with the appearance of intracellular microcolonies, indicating that hepatocytes may serve as a niche for *E. faecalis*. Whether the intracellular lifestyle of *E. faecalis* in hepatocytes contributes to liver injury remains to be investigated. Combining cellular models of infection, bacterial genetic screening and pharmacological agents to modulate host-signalling pathways, we identified bacterial candidates involved in invasion or intracellular multiplication of *E. faecalis* in hepatocytes. We also showed that lipid droplets support *E. faecalis* intracellular multiplication in hepatocytes. Finally, we identified an *E. faecalis* transcriptional regulator involved in regulating the level of lipid droplets. We anticipate this work will provide insights into the contribution of intracellular *E. faecalis* growth in liver injury.

**Role of the CckA-ChpT-DivL complex in the phosphorylation of the master regulator CtrA during the cell cycle and nitrogen-fixing symbiosis in *Sinorhizobium meliloti***

Mohammedi Roza

*Sinorhizobium meliloti* is an alphaproteobacterium which is able to live free in the soil or in symbiosis with legumes. During symbiosis, bacteria fix atmospheric nitrogen within symbiotic organs, called nodules, where they undergo extreme cell differentiation into bacteroid. Bacteroids are characterized by genome endoreduplication, cell enlargement and high membrane permeability. The transcription factor CtrA has been shown to be the master regulator of the cell cycle and the transition from a free to a symbiotic lifestyle is accompanied by a gradual disappearance of CtrA during the differentiation of bacteroids, suggesting that the (de-) regulation of the cell cycle by CtrA is a crucial point for the establishment of the symbiosis. In the alphaproteobacterium *Caulobacter crescentus*, a bacterium related to *S. meliloti*, cell differentiation is also closely related to the cell cycle via the activity of the master regulator CtrA. CtrA has been shown to be activated by phosphorylation via a phosphorelay system consisting of the two histidine kinases DivL and CckA and the histidine phosphotransferase ChpT. Orthologs of these different regulators are present in *S. meliloti*, suggesting a conservation of this module in the regulation of CtrA in this bacterium.

The objective of this work is to study the functions of the CckA-ChpT-DivL complex and its impact on CtrA in *S. meliloti* in free and symbiotic life. We first confirmed the essentiality of *divL* in *S. meliloti* by transduction of a deletion cassette. The study of a DivL-depletion strain allowed us to demonstrate that DivL is essential for the proper functioning of the cell cycle and that it is involved in the regulation of CtrA. A translational fusion with the fluorescent protein YFP showed that DivL is localized to a single pole. We also purified the phosphorelais proteins and reconstructed a part of the phosphorylation cascade in-vitro. Finally, the DivL-depletion strain is not able to perform an efficient symbiotic relationship with *Medicago sativa* under the tested conditions.

## Single-step upgrade and desulfurisation of biogas by microbial electrosynthesis – the DUB.ME Biomethane project

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To meet growing energy demands sustainably, economies need to prioritize bioenergy and biogas from waste materials. The potential of biogas and biomethane production from sustainable feedstocks, including animal manure, remains currently untapped[1]. Looking into the future, the availability of agro-industrial feedstocks that could be directed to biogas and biomethane is expected to increase by 40% by 2040[1]. Biogas from such feedstocks must undergo significant desulfurization and upgrading to enable its injection into the gas grid as the $H_2S$ content can reach a few thousand ppm and $CO_2$ is still present by 25 to 55% v/v. The upgrading technologies implemented thus far at industrial scale are energy intensive, can suffer from high maintenance costs, while they emit the $CO_2$, removed from the biogas back to the atmosphere[2]. As an alternative, biological processes that rely on chemoautotrophic, hydrogenotrophic microorganisms to convert $CO_2$ to $CH_4$ have been proposed. A microbial electrochemical route has emerged the last ten years for methane production, often referred to as ‘‘electromethanogenesis’’.

In electromethanogenesis, the typical electrolysis cell is employed, where the reduction of $CO_2$ to $CH_4$ is catalyzed by electroactive microorganisms, that source reducing power either directly from a solid conductor, the cathode, or indirectly, from $H_2$[3]. Thus the process is referred to as microbial electrosynthesis (MES). In MES the $CO_2$ content of biogas can be reduced to $CH_4$ by biocathodic communities, aiding as such the upgrade to biomethane. During these last ten years a great amount of publications emerged and production rates as high as 12.5 $L \cdot L^{-1} \cdot d^{-1}$ and 141 $L \cdot m^{-2} \cdot d$ have been obtained[3]. Still, efforts need to be undertaken to render reduction rates more appealing to scale-up and increase the energy efficiency of the process. Although $H_2S$ is a common hazard that needs to be removed from biogas, interestingly, $H_2S$ oxidation offers a thermodynamic advantage due to its lower electrode potential[4]. In fact, a large sum of research has been dedicated to sulfide oxidation reaction (SOR) coupled to the $H_2$ evolution reaction (HER), to provide more energy efficient electrolysis compared to the oxygen evolution reaction (OER) (0.17 V vs 1.23 theoretical cell voltage)[5]. Coupling $H_2S$ oxidation to $H_2O$ or $CO_2$ reduction could pose an alternative, more energetically favourable option for biogas upgrading.

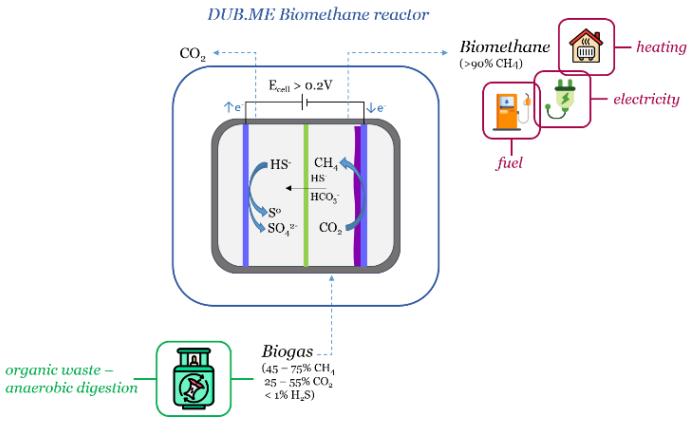


*Figure 1 - DUB.ME Biomethane processes: A single microbial electrolysis (MES) cell, to aid biogas desulfurization and upgrade to ultimately transform biogas to biomethane*

With this project we aim to achieve different objectives. Firstly, to suggest implementation of a single system for biomethanation without the need of external $H_2$ and which additionally eliminates the need for a pre-upgrade biogas desulfurization. Furthermore, to propose a sustainable alternative to catalytic methanation by replacing expensive catalysts with a biocatalyst capable of reducing $CO_2$ to $CH_4$ and furthermore, capable of tolerating higher concentrations of $H_2S$. Besides, we aim at a less energy-intensive approach to bioelectrochemical methanation by utilizing, partially, the sulfide oxidation reaction (SOR) as the anodic reaction instead of the traditional oxygen evolution reaction (OER).

To execute this project we are looking for an M2 intern to assist in first step bioelectrochemical, lab-scale, cell construction and sulfide toxicity investigation, as well as in the development of an advanced method for sulfur ions analysis. Additionally, a 2-year postdoctoral researcher will be recruited to undertake the development of a larger-scale bioelectrochemical reactor as well as to work on the development of alternative anode materials.


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# SPECIALIZED METABOLITES WITH ANTIVIRAL PROPERTIES FROM A VIETNAMESE PLANT: ISOLATION AND SYNTHESIS

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The plant metabolites group at the Institute of Chemistry of Natural Substances (ICSN) aims to study and valorize specialized metabolites isolated from plants. For that, the plant extract library named Extractothèque ICSN, containing more than 15,000 extracts, is regularly screened by biologist partners. Recently, a selection of 1650 plant extracts from Extractothèque ICSN was evaluated on an inhibition cell-based assay on human Coronavirus (HCoV-229E and SARS-CoV-2). The EtOAc and MeOH extracts of the leaves of the Vietnamese species *Melodorum fruticosum* were selected for their good activity on HCoV-229E. Bio-guided fractionation led to the isolation of 14 pure products, including 3 novel compounds. Their antiviral evaluation confirmed the interest of some metabolites with $IC_{50}$ around 1.8 µM for toussaintine C. To validate their activity and improve our knowledge of the structure-activity relationships, we performed the total synthesis of toussaintine C and developed a divergent synthesis of analogs.

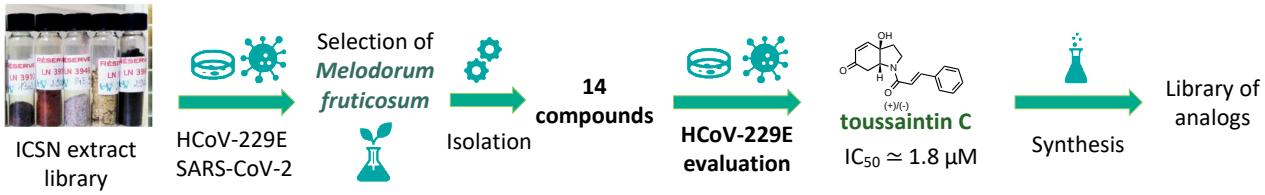


Figure 1: The scheme of the project procedure

# A new electrochemical genosensor for detection of tetracycline and β-lactams resistance genes: focus on *Staphylococcus aureus* and *Escherichia coli* isolated from milk and meat in Italy

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Antimicrobial Resistance (AMR) is an alarming threat to human health. According to the World Health Organization (WHO), 1.27 million deaths were attributed to AMR in 2019.
The transmission of AMR along the food chain, starting from raw materials to final products, plays a plausible role in spreading various AMR bacteria, including *Staphylococcus aureus* and *Escherichia coli*. The potential transmission of antibiotic-resistance genes (ARGs) through contaminated food consumption elevates the risk for final consumers, establishing a potential pathway for entry into humans. Conventional AMR surveillance relies on isolating indicator microorganisms, followed by phenotypic characterization and antimicrobial susceptibility testing (AST). However, this approach is time-consuming and has high costs. Consequently, there is an urgent need for rapid and specific methods to detect ARGs. Biosensors could be a key solution, in particular the electrochemical genosensor for specific ARG detection in food matrices, due to their high specificity, short time response, and practical applications.

This study focused on *S. aureus* and *E. coli* isolated from raw milk and meat in Italy and tested for their resistance against antibiotics based on the EUCAST instructions. The results showed prevailing AMR to tetracycline and gentamicin observed in *S. aureus* and to ampicillin in *E. coli* strains. Specific primers for ARGs responsible for these AMRs were utilized in multiplex PCRs on the isolated strains, to detect their presence. The ARG DNA sequences were then aligned and used as targets to design new ss-DNA probes intended for use in the electrochemical genosensor. Two main targets were chosen: *tetK* and *blaTEM* genes, the most present tetracycline and β-lactams resistant genes in the bacterial isolates. The specificity of the probes was evaluated *in silico* and it was confirmed through the dot blot procedure and the outcomes related to the electrochemical genosensor using the new ss-DNA probe specific for the *tetK* gene established a sensitivity of 100fg/μL within a hybridization time of 30 minutes. This analysis provides a starting point to detect the presence of ARGs quickly and with low costs in bacteria isolated from food matrices.

# Determining the main causes of phage resistance in clinical strains of *Pseudomonas aeruginosa*.

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A current challenge for the development of phage therapy is to find the right phages targeting the strains responsible for a patient's infection. Consequently, much effort is made to constitute a broad portfolio of phages with different activity spectrum. A second concern is the emergence of phage resistance, which relies on mechanisms ranging from the blockage of phage adsorption to a variety of intracellular defenses. Bacteria can be intrinsically resistant to specific phages if they lack their receptors or if they carry defense systems that are active against them. When resistance does not pre-exist, it can rapidly evolve upon phage exposure.

Our objective is to determine the landscape of inherent resistance in a panel of 125 clinical strains of the opportunistic pathogen *Pseudomonas aeruginosa* associated with antibiotic treatment failure. A collection of 9 anti-*Pseudomonas aeruginosa* phages, belonging to 6 different genera and exhibiting receptor varieties, has been used to build an interaction matrix: a phage susceptibility test, which include a microdilution assay and a plaque assay, was performed on each strain to assess phage activity *in vitro*. When a strain was resistant to a phage, the underlying mechanisms were studied. First, we investigated whether phage resistance resulted from impaired adsorption and when this was not the case, the genomes of the strains were sequenced to identify potential intracellular mechanisms of phage resistance. We next aim to determine whether strains carrying multiple defense systems, or specific system combinations, have higher overall phage resistance.

These results allow to estimate the prevalence of intrinsic resistance, and its underlying causes, in clinically relevant isolates. They will contribute to improve the design of future phage discovery and cocktail assembly strategies and help to mitigate the impact of phage resistance on therapy outcomes.

# Tolerance to the antifungal drug fluconazole is associated with increased cytoplasmic crowding/viscosity

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*Candida albicans* is an opportunistic human fungal pathogen that is a major cause of life-threatening nosocomial infections, as well as persistent mucosal infections. This fungus is one of four fungal pathogens listed in the Critical Priority Group highlighted by the World Health Organization in 2022 [1]. Currently, four main classes of antifungal drugs, polyenes, azoles, allylamines and echinocandins, are used to treat fungal infections and, as a result, drug resistance or tolerance can frequently limit treatment outcomes. Antifungal drug resistance occurs when a genetic advantage enables survival at an otherwise inhibitory drug concentration. However, in many clinical isolates, a portion of the cell population exhibits slow growth above the minimal inhibitory concentration (MIC) [2, 3]. This subpopulation, referred to as tolerant cells, has been linked to recurrent and persistent fungal infections, such as those due to *C. albicans.*

Cellular reactions can be affected by the physical properties of the cytoplasm [4]. Hence, we have been investigating the consequence of prolonged exposure to supra-MIC antifungal drugs, such as the widely used fungistatic drug fluconazole, on the physical properties of the *C. albicans* cytoplasm. To examine the cytoplasmic crowding/viscosity, we have optimized a micro-rheological probe, consisting of genetically encoded multimeric nanoparticles (GEMs) [5]. Our results indicate a substantial increase in cytoplasmic crowding/viscosity upon prolonged exposure to high levels of antifungal drugs that inhibit ergosterol biosynthesis, such as fluconazole. To confirm that the effect on cytoplasmic crowding/viscosity is due to the inhibition of the fluconazole drug target lanosterol 14-alpha-demethylase Erg11, we carried out complementary studies in *erg11* mutants. Upon repression of *ERG11* expression, a similar increase in cytoplasmic crowding/viscosity was observed. We hypothesize that these changes in cytoplasmic crowding/viscosity are due to increased levels of ribosomes, as inhibition of ribosome biogenesis restores the effective diffusion of the GEMs in the presence of fluconazole. Taken together, our results suggest that the changes in the physical properties of the fungal cytoplasm, upon inhibition of ergosterol biosynthesis, promote slow-growth that is critical during drug tolerance.

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**Study of the genetic competence development in Staphylococcus aureus using the firefly luciferase as a reporter gene**

**Poirette Pierre**

Nowadays, emergence of antibiotic resistances has become one of the most important public health concerns, sometimes defined as a “hidden pandemic”. *Staphylococcus aureus*, known as the most lethal bacterial pathogen in France has demonstrated its ability to accumulate antibiotic resistances. The emergence of multi-drug resistant strains is highly correlated with their capacity to perform natural transformation (NT), one of the three main HGT mechanisms. Interestingly, NT is entirely controlled by the recipient cell that needs to enter a genetically encoded differentiated state called genetic competence, that has recently been demonstrated in *S. aureus*. Genetic competence is a sequential and transient process, tightly regulated by the expression of successive actors among which are found SigH and ComK1, two key central competence regulators. Recently, we found that genetic competence is induced by an oxygen starvation (*i.e*., microaerobic conditions) that may be sensed by the two-component system SrrAB which is thought to activate SigH. Here, taking profit of the characteristics of the firefly luciferase used as a powerful transcriptional reporter we were able to study, in detail, the genetic competence development program.

**Title: Development of Smart Lipid-Porphyrin Nanoassemblies for Enhanced Photothermal and Photodynamic Therapy: A Novel Approach to Combat Antibiotic-Resistant Bacterial Infections**

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The escalating global threat posed by antibiotic-resistant bacteria and their resilient biofilms demands innovative therapeutic strategies. In Europe, antimicrobial-resistant infections claim over 35,000 lives annually,[1] and without intervention, the World Health Organization (WHO) predicts a surge in deaths from drug-resistant bacteria, reaching 10 million per year by 2050.[2,3] Addressing this critical challenge requires the development of advanced antibacterial approaches capable of overcoming resistance mechanisms.

The eradication of microorganisms by reactive oxygen species and mild hyperthermia can be induced by administering photoactive materials. Thus, photodynamic therapy (PDT) and photothermal therapy (PTT) based on lipid-porphyrin (PL-Por) conjugates have recently emerged as new promising antimicrobial treatment modalities for bacteria and biofilm eradication in localized infections.[4]

In this study, novel supramolecular nanotherapeutic platform was presented aiming at combating bacterial infections and biofilms. The introduced approach includes designing of nanoplatform exhibiting photothermal and photodynamic properties through the generation of heat and reactive oxygen species (ROS) in a controlled manner. The nanoassemblies were constructed by conjugation of natural lipids to photosensitizers such as porphyrins via a smart linker that lead to the synthesis of smart lipid-porphyrin conjugates, facilitating self-assembly into liposomal structures with an unprecedentedly high porphyrin payload. A key innovation lies in the controlled release of the porphyrin moieties upon illumination, thus leading to improved and controlled PTT/PDT properties.

The photoinduced bactericidal efficacy of these supramolecular assemblies was systematically evaluated by *in vitro* tests on planktonic bacteria cultures of Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria by Colony Forming Unit (CFU) assays. Results demonstrated a remarkable antibacterial effect against both strains. Staphylococcus aureus strains were completely eradicated with as low as 0.01 µM concentration of photosensitizer present in the assemblies, while 10 µM concentration was needed to obliterate nearly 90% of *Pseudomonas aeruginosa* that is greatly linked to its lower sensitivity to PDT.[5]

Furthermore, self-assemblies incorporating the new lipid-porphyrin conjugates exhibited a two-order-of-magnitude and two-fold increase in efficiency for Staphylococcus and Pseudomonas strains, respectively compared to conventional conjugates.

The preliminary results highlighted the robust antibacterial effect of the new PL-Por formulations. Future efforts will focus on enhancing targeting properties by decorating the liposomes with specific targeting moieties, offering a promising avenue for the development of precision antibacterial therapies. This work signifies an advancement in the pursuit of effective treatments against antibiotic-resistant bacterial infections.

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The development and optimization of a bioproduction process require a predictive growth model. Existing models, grounded in empirical laws, exhibit limited predictive capability. The Microbial Transition State (MTS) model, employing a thermokinetic approach rooted in statistical physics, establishes a link between growth kinetics and the energy balance of cell metabolism. Notably, this model has demonstrated success in simulating both microbial communities and pure cultures. Further exploration of the MTS theory is essential to understand the impact of environmental conditions on growth dynamics.

**Combined effect of genetics and intestinal microbiota on variability in vaccine response in laying hens**

Authors

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Vaccination is the most effective strategy for preventing infectious diseases in livestock, however the efficacy of most vaccines varies. Genetic variations in the host and in the gut microbiota are two of the factors that can affect this efficacy[1]; studying their combined effect on vaccine response is an integrated and innovative strategy. As part of this project, we will first focus on the changes in the gut microbiota following various perturbations so that we can then correlate it with the variability in vaccine response. We studied the caecal microbiota composition of: (1) a strong perturbation of the gut microbiota through the ingestion of antibiotics, (2) a change in the rearing environment and (3) the genetic line. To this aim, we compared 4 groups (n=50 per group) of two chicken lines (Rhode Island red (RIR) and Leghorn (LEG)), treated or not with a cocktail of 3 antibiotics and reared under different conditions (fully indoor or with outdoor access). The gut microbiota was sampled at 5 different times during the experiment, which lasted 24 weeks. Individual microbiota compositions and diversities at different timepoints were assessed through a bio-informatic analysis of amplified sequences of the 16S rRNA gene. From our results, our main conclusions are: (1) antibiotic treatment altered the gut microbiota of both lines, with a line effect; (2) the combined effect of antibiotic treatment and housing altered the gut microbiota of the two lines; and (3) the microbiota of the outdoor treated group was closer to the untreated group than the indoor treated group. For the future, we will analyze how genetic variations at the individual level in laying hens are related to both vaccine response and gut microbiota variability.

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# TOWARDS NEW NATURAL CARBAPENEMASE INHIBITORS

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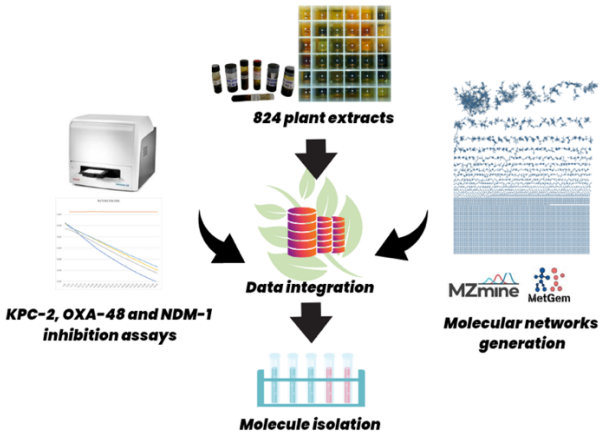
The threat of antibiotic resistance is more than ever present in our society and will soon become the main cause of death worldwide. Three carbapenem-resistant bacteria are classified by the World Health Organization (WHO) among the most compelling in the race to develop new therapeutic solutions. Carbapenemases are a type of enzymes synthetized by a large number of these bacteria which hydrolyze the beta-lactame core of the antibiotic structure, thus inactivating it. This represents nowadays one of the main mechanisms responsible for the degradation of this type of large spectrum antibiotics.

In this context, a library of 824 plant extracts from rich biodiversity world areas (Malaysia, Vietnam, and New Caledonia) was screened against three of the main existing carbapenemases (KPC-2, OXA-48, NDM-1) to find natural inhibitors of these enzymes. The method employed for this screening is based on a measure of the decrease in the optical density of imipenem (a carbapenem antibiotic) when adding the hydrolyzing enzyme. This decrease is slowed or interrupted in the presence of a carbapenemase inhibitor.

This screening ended up in the selection of five plant extracts with high and reproductible activity profiles inhibiting two of the three tested carbapenemases. Among this selection, *Fissistigma litseaefolium,* a Malaysian climber plant species, was selected for further analysis, due to the high activity showed by both bark and leaf extracts. A bioactivity and mass-guided isolation of the potentially active molecules, combined with molecular networking analysis to obtain a clear visualization of taxonomical data and distribution of the activity between species has been conducted, as well as a phytochemical study of this unstudied plant.



## Unravelling complex interactions in fluctuating habitats

Redaelli Tommaso

In nature, microbes commonly thrive in large communities linked by a **complex network of interactions**[1]. The outcomes of such interactions shape the dynamics of life on Earth and significantly affect environmental and human health, as well as climate stability[2]. The interactions underlying bacterial communities drive but also respond to environmental shifts in a feedback behaviour due to the responsive nature of bacterial communities[3]. Microbial habitats are inherently dynamic, constantly pervaded by **environmental fluctuations** across a wide range of **temporal scales**[4]. For instance, marine microbes experience environmental fluctuations on a daily scale due to sunlight cycles[5]. In soil, plant roots release nutrients in dynamic bursts, causing rapid changes in microbial nutrient availability within minutes[6]. Moreover, wetting and drying cycles in soil, driven by factors like hourly rainfall frequency and monthly seasonal variations, lead to fluctuations in nutrient concentrations[7]. Bacteria within mammalian guts encounter fluctuations in nutrient concentrations corresponding to the frequency of meals consumed by the host[8]. **How do the timescales of the environmental fluctuations influence the bacterial community dynamics?** This question delves into a crucial ecological challenge, exploring the impact of environmental fluctuations on the interaction dynamics within microbial communities.

Microbial communities are typically complex systems consisting of thousands to billions of individual cells belonging to tens to hundreds of different species. Within communities, interactions can be inter- or intra-species, pairwise, i.e. involving two species, or higher-order, i.e. establishing relationships between ensembles of three or more species. An ongoing debate in ecology revolves around whether multi-species interactions can be explained as linear combinations of pairwise interactions[9] or should be understood as complex nonlinear processes[10]. The majority of microbial interactions research has been carried out for pairwise interactions under steady conditions or following single-step alterations in nutrient concentration. Recent findings indicate that in pairwise communities, interactions may vary on timescales much longer than the timescales of recurrent fluctuations[11]. The way recurrent environmental fluctuations timescales affect the state, maintenance, and spatio-temporal dynamics ("trajectories") of more complex microbial communities, of three or more species, remains entirely unexplored. In this talk, I will explain first results I obtained in the directions of covering this knowledge gap. I quantified numerically, both in spatial and temporal dimensions, the effects of recurrent environmental fluctuations on bacterial higher-order interactions. I explored the dynamics of essential interaction parameters such as cells leak, uptake and growth rates. I observed the spatial organization of a 3 species community within a fluctuating chemical landscape.

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# Selection, adaptation and characterization of electrosynthetic microbial communities

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In the context of the energy transition, the use of organic waste in environmental biorefineries is an attractive option due to its low cost and potential to replace fossil fuels. However, this approach presents technological challenges due to the heterogeneity and variability over time of organic waste, increasing the complexity of purification treatments for the molecules of interest and resulting in higher final costs. Microbial electrochemical technologies are emerging as promising solutions to overcome these problems, by allowing the physical separation of oxidation from contaminated waste streams used as raw materials (bioanode) from the synthesis of bio-based chemical molecules (biocathode). Recent scientific studies have focused on the production of methane and acetic acid at the cathode, associated with the electrolysis of water at the anode (abiotic).

The objective of this study is to combine a bioanode with a biocathode, in order to reduce carbon dioxide to carboxylates, by the selection of homoacetogenic bacterial communities, at the cathode. The electrons generated at the anode, by oxidation of the organic matter by electrogenic microorganisms, are transferred to the cathode, for the reduction of carbon dioxide into multicarbon molecules by electrotrophic microorganisms. In addition to the two main compartments, an intermediate compartment, isolated by ion exchange membranes, is integrated in order to extract and concentrate the carboxylates produced in a sterile solution. This research aims to explore the diversity of carboxylates produced at the biocathode as a function of the selection and dispersion conditions of the microbial assembly.

Both compartments are inoculated with the same mixture of biowaste hydrolysate (Tian et al., 2023) and salt marsh sediments, as hypersaline inocula have been identified as particularly suitable for microbial electrochemical applications. These inoculation conditions make it possible to enrich microbial diversity and study the selection process that takes place at the anode and cathode on the same reservoir of diversity. Only the mixture intended for cathode inoculation is heat-treated in order to select homoacetogenic bacterial communities capable of sporulation, from the class *Clostridia* (Diallo et al., 2021). Anaerobic conditions are ensured by $N_2$ bubbles and the cathode is supplied daily with $CO_2$, which is the only source of external carbon. In addition, the two electrodes have distinct geometrics: the anode is a carbon cloth while the cathode is a carbon brush with granules of the same material. Thus, the same inoculum is subject to different selection processes, including oxidation or reduction capacity, different carbon sources, the ability to form a biofilm and to carry out electron exchanges with a 2D electrode or 3D, etc.

The main objective of this first triplicata experiment is to evaluate the efficiency of the use of a hypersaline inoculum, both for the oxidation of organic matter at the anode, and for the reduction of carbon dioxide to carboxylates at the cathode, as well as the efficiency of the extraction of these carboxylate ions in an intermediate compartment. We also want to determine all the carboxylates produced, in order to evaluate the possibility of diversifying or, on the contrary, of specializing the synthesis of these molecules by modifying the microbial assembly through selection and dispersion.

Ultimately, this project aims to guide the production of dyes by a model microorganism, froms carboxylates formed in the biocathode and concentrated within the intermediate compartment. These dyes will have specific applications in the textile industry, demonstrating the potential of this innovative approach in the development of sustainable processes for industrial applications.

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Title: Iron acquisition mechanisms as a factor in structuring microbial communities on cheese surfaces

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Microbial communities on the surface of cheeses are composed of a wide variety of interacting micro-organisms. The structure of these communities, composed of inoculated (ferments) and non-inoculated (from the environment) microbial strains, is still only partially understood. Several features indicate that interactions via iron acquisition mechanisms play a role in the structuration: i) the presence of many iron acquisition genes in some bacteria of these communities, ii) the identification of mutualistic interactions linked to iron metabolism. This thesis aims to identify new biotic interactions via iron acquisition mechanisms in these communities by exploring these functions in metagenomic and genomic data from 44 French PDO cheeses. For this, we searched for specific motifs (HMMs) of genes involved in the production and transport of siderophores (metabolites capable of capturing iron from the environment). These results enable us to propose potential interactions, positive or negative, based on complementary capacities (synthesis-transport) between microbial species from cheese surface. Growth tests and transcript analysis in model environments will validate these hypotheses. Knowledge of these interactions will help us to better understand the structuring of microbial communities on cheese surfaces. Ultimately, these results could enable us to design ripening ferments with optimal colonizing capacity and propose strategies for limiting the implantation of undesirable micro-organisms in cheeses.

**Cutaneous leishmaniasis therapy with novel Amphotericin B liposomal Formulation**

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Leishmaniasis, a group of neglected tropical diseases, predominantly affects socially vulnerable populations with limited access to healthcare, often involving immunocompromised individuals. The disease, caused by various Leishmania species, manifests primarily as cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). In 2022, there were 205,986 and 12,842 new cases of CL and VL, respectively (1). Among the few drugs available, amphotericin B (AmB) is considered the most potent antileishmanial. Despite its effectiveness, AmB faces challenges such as low solubility, high molecular weight, and self-aggregation, leading to reduced bioavailability and increased toxicity (2). Liposomal AmB, or AmBisome®, is considered highly effective, yet limitations exist, especially in complicated CL cases and HIV/VL coinfected patients. This study introduces innovative AmB-PEGylated liposomes (LAmB) for CL therapy, demonstrating favorable characteristics, including a hydrodynamic diameter of 128.4 ± 4.8 nm, low polydispersity index of 0.10 ± 0.02, and slightly negative surface charge -3.6 ± 0.6 mV, suitable for *in vivo* administration. A high encapsulation rate (94.8 ± 5.2%) was also obtained. The physicochemical characteristics of LAmB remained stable over 30 days in the refrigerator. Additionally, the aggregation state of AmB in the formulation was stable over time. Furthermore, LAmB demonstrates lower hemolytic activity compared to the commercial formulation Anforicin B®. In a murine model infected with *L. (L.) amazonensis*, LAmB treatment leads to a marked reduction in lesion size (8.77 ± 1.60 mm) in comparison to the untreated control (11.32 ± 1.70 mm) at the end of treatment. Notably, the LAmB group had smaller lesion size than AmBisome® (9.87 ± 1.85 mm). This novel formulation emerges as a promising therapeutic approach for CL.

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**From microstructure development to quality changes and viral risk: multiscale analysis of frozen raspberries.**

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## Abstract

Quality properties of frozen foods are strongly related to the product microstructural organization such as ice crystal structures. Ice crystals characteristics are defined both by the freezing process and the frozen storage conditions. For example, fluctuating storage temperatures cause ice crystals growth by recrystallization, leading to tissue damages and subsequent quality losses. Microstructure imaging techniques such as X-ray microtomography could be useful for a better understanding of the complex mechanisms that take place at the microscopic level in order to reduce macroscopic quality changes during frozen storage.
Raspberries are known for their health benefits but they are difficult to preserve for a long time. Freezing process is usually used to increase their shelf life, but these fruits are highly sensitive and encountered freeze damages. On a second hand, frozen raspberries are known to be responsible of foodborne diseases with the transmission of enteric viruses such as Hepatitis A virus (HAV). Cultivated, harvested and frozen in developing countries where the HAV is endemic, contaminated raspberries are imported in developed countries in which the virus is an emerging concern. HAV infections occur mainly by the faecal-oral route by the consumption of contaminated food or water (Hu, 2020) and 2 to 7% of worldwide HAV outbreaks are due to HAV contaminated food ingestion (Randazzo, 2020). If HAV is known to resist freezing, the impact of the freezing process on its persistence is not established yet.

In this work, X-Ray microtomography was used to investigate microstructural changes occurring during freezing and storage of raspberries under different conditions. The fruits were imaged directly at the frozen state thanks to a cooling stage (-20°C). The analysis of X-ray images showed ice crystal growth with storage temperature, temperature fluctuations and storage duration. Quality parameters, such as texture and drip loss, were also measured. Raspberries texture, along with drip loss, was altered by freezing and during storage, probably due to cell perforation caused by ice crystals formation and growth. Artificially contaminated raspberries were submitted to different conditions of freezing and storage. Infectious HAV were quantified at different times during the storage by cell culture using Real-Time Cell Analysis assay. The obtained results suggest that the higher the temperature of storage is, the lower the HAV persists on raspberries.
These results taken together, show how microstructural changes can affect macroscopical quality parameters and the persistence of infectious viruses.

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# Poster title: Towards the structural characterization of MAM (Microbial Anti-inflammatory Molecule) from *Faecalibacterium*

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The genus *Faecalibacterium* is one of the most abundant bacteria in the intestinal microbiota, with species related to gut protection and the promotion of health. The anti-inflammatory properties of this bacteria come from several aspects, such as metabolite production and active peptides in the supernatant that belongs to MAM (Microbial Anti-inflammatory Molecule), a unique protein expressed by this genus. Although the anti-inflammatory activity of MAM has been already shown through in vivo and in vivo studies, its role in the Faecalibacterium is still unknown. This work aims to characterize the protein MAM from different species of the genus. To do that, MAM's structure was predicted using AlphaFold. Despite the models being very different, as we expected due to the sequence diversity, a main helix core is conserved among the species. Further, disorder regions are present in all predicted structures, indicating flexibility regions. As the gene of the ABC transporter with a peptidase domain is conserved in the genetic environment of MAM, after its structural prediction, the Molecular Docking (MD) was performed between the two proteins using AlphaFold. Our results reveal strong evidence that the most conserved region of MAM, the N-terminus, interacts with the peptidase domain of the ABC transporter, indicating that MAM can be cleaved and exported through the ABC transporter. Also, to understand the subcellular localization of MAM, LC-MSMS proteomic analysis was performed, and it was observed that the majority of MAM belongs to the insoluble fraction, which corresponds to the cell wall/membrane proteins.

# Mode of action of marginolactone on bacterial membranes

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Azalomycin F (AzaF) is a marginolactone produced by *Streptococcus* spp [1, 2]. While its antimicrobial properties against gram-positive bacteria are known, its exact mode of action is not clear yet [1]. This would be important to understand the extent to which marginolactones can be used in clinical applications. One hypothesis is that it might cause damage of the cell membrane [3]. We therefore investigated the interaction of AzaF and other marginolactone with model membranes and bacterial cultures.

We found that the growth of several bacteria is significantly negatively affected by different concentrations of AzaF. Furthermore, we assessed the interaction of different amounts of AzaF and other marginolactone on lipid vesicles via time course fluorescence spectroscopy and microscopy.

The results demonstrate the influence on lipid membranes at different concentrations of marginolactone, contributing to understanding the mode of action of newly discovered natural products on their antimicrobial mode of action.

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**Title**
SARS-CoV-2 Orf9b: Effects on mitochondrial physiology

**Authors (underline the presenting author)**
Yining ZHAO, ElodieVIMONT, Genny AMADEI , Timothy WAI

**Abstract (max 300 words)**
Mitochondria have primarily been viewed as bioenergetic and biosynthetic organelles that autonomously co-exist within the cell but they also act as signaling organelles that play a role in cellular stress responses. Under stress conditions, the outer membrane can become permeable, leading to the release of pro-apoptotic factors and pro-inflammatory molecules that activate inflammatory signaling and cytokine secretion.

SARS-CoV-2, can infiltrate cellular defense mechanisms, including those mediated by mitochondria, to evade the host immune response. The SARS-CoV-2 genome encodes 27 proteins, one of which, Orf9b, physically and functionally interacts with mitochondria via binding to TOM70, a mitochondrial outer membrane protein. Orf9b has been shown to negatively impact innate immune signaling and its recruitment to mitochondria requires phosphorylation of specific serine residues. However, it is unclear how Orf9b influences other critical mitochondrial functions.

My project is to investigate the impact of Orf9b on various mitochondrial including oxidative phosphorylation, mitochondrial fission and fusion, regulated and programmed cell death, protein import, mitophagy, and innate immune signaling. I also plan to investigate how Orf9b recruitment is regulated and the mechanisms that lead to its degradation when recruitment is inhibited. We may also find novel Orf9b receptors of the outer mitochondrial membrane involved in Orf9b recruitment. The successful execution of my project would provide insights into the process of Orf9b binding to mitochondria and its impact on mitochondrial and cell physiology. It may also shed light on potential therapeutic targets for COVID-19 and other viral diseases that exploit mitochondria-mediated cellular defense mechanisms.

**Key words (max 5)**
Mitochondria
SARS-CoV-2
Orf9b
Mitochondrial functions
Protein recruitment

## Beyond Vaccinia: Exploring the Structural Diversity of Poxviruses

Zimmeck Marcel

Poxviruses represent a large family of dsDNA viruses that can pose a significant threat to public health due to their ability to infect a broad range of hosts and spread in human populations. Vaccinia virus (VACV) has become a model to study poxvirus biology and pathogenicity. However, the diversity of this viral family remains overlooked and knowledge about prominent members from genus other than *Orthopoxvirus* is critically lacking. My PhD project combines state-of-the-art cryo electron tomography (cryoET) and advanced subvolume averaging processing techniques to unravel the intricate structural details of infectious particles produced by different poxviruses. Our ultimate goal is to improve our understanding of the morphology, composition, and genomic characteristics of viruses from different genus that have epidemic potential. For this, we are analyzing native poxvirus samples of purified infectious particles in a biological safety level 3 environment, utilizing robust pipeline for sample preparation, data acquisition and data processing using the IMOD suite, coupled with subvolume averaging facilitated by PEET. In particular, recent studies looking at the prominent palisade layer on the core surface of VACV have revealed its composition as densely packed trimers of the major core protein A10. One aspect of the project seeks to explore if such structural characteristics are similar in the viral cores of other poxviruses. Altogether, our expected findings are likely to provide key and novel insights into the structural diversity of poxviruses, and hence a foundation for the development of targeted antiviral strategies that are currently missing. By elucidating virion structures at high resolution and in the native environment, we aim at increasing our understanding of poxvirus diversity and pathogenicity, which is crucial to improve our preparedness and response against potential outbreaks in the dynamic landscape of emerging and re-emerging infectious diseases.

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