Comité d'organisation

Sophie Abby (TIMC)

Sylvie Elsen (IBS)

Pierre Marcoux (CEA)

Comité scientifique

Joël Gaffé (TIMC)

Thomas Hindré (TIMC)

Ivan Junier (TIMC)

Corinne Mercier (TIMC)

Audrey Le Gouellec (TIMC)

Un grand merci à nos sponsors pour leur soutien financier :

Le Labex GRAL, l'Université Grenoble Alpes, la fédération OLIMPICS, le laboratoire TIMC et la Métropole Grenoble-Alpes

Pour vous inscrire à la mailing list et recevoir les informations de BactoGre, envoyez un mail à Sophie Abby

BactoGre 2025 - Programme

Auditorium de l'IMAG - Mardi 27 mai 2025

8:30 Café d'accueil & Installation des posters (Hall d'accueil)

9:00-10:30 Modérateurs : **Audrey Le Gouellec & Thomas Hindré**

- **9:00 Accueil**
- **9:05 Sam Meyer** *"Modeling global transcriptional regulation by DNA supercoiling in bacteria"*
- **9:50 Marc Joyeux** *"Associative and segregative phase separation cooperate in organizing the bacterial nucleoid"*
- **10:10 Irina Mihalcescu** *"Quantitative study of low concentrations of DnaA in* Escherichia coli*, using the* uhp *expression system"*

10:30 Pause-café + séance posters

11:00-12:20 Modérateurs : **Sophie Abby & Joel Gaffé**

- **11:00 Corinne Mercier** *"Long bacterial cell" phenotype in the Long-Term Evolution Experiment (LTEE) on* Escherichia coli*, a selective advantage?"*
- **11:20 Audrey Le Gouellec** "*From Metabolomics to Transcriptomics: Deciphering Polyamine Regulation Mechanisms in* P. aeruginosa *during Chronic CF Infection"*
- **11:40 Eva Gendron "***Dynamics of bacterial populations and their antibiotic resistance profiles in hospital wastewater from an intensive care unit"*
- **12:00 Aurélien Tauzin "***Estimating bacterial mutation rates with simulation-based methods (ABC)"*

12:20 Déjeuner et posters

14:00-15:25 Modérateurs : **Corinne Mercier & Ivan Junier**

- **14:00 Catherine Larose** *"Microbial communities in glacier environments: Life below freezing"*
- **14:45 Sophie Abby** "*Dynamic quinone repertoire accompanied the diversification of energy metabolism in Pseudomonadota"*
- **15:05 Timothée Salzat-Hervouette** "*Investigating the evolution of phototrophy in Pseudomonadota"*

15:25 Pause-café + séance posters

15:55-16:35 Modérateurs : **Sylvie Elsen & Pierre Marcoux**

- **15:55 Eugenio Cinquemani** *"Characterization and control of microbial consortia on an automated mini-bioreactor platform"*
- **16:15 Jochen Fick** *"Optical trapping and swimming analysis of* Pseudomonas *bacteria"*

16:35 Conclusion et clôture de la journée

Résumés des présentations orales

Sam MEYER – MAP, INSA Lyon

"Modeling global transcriptional regulation by DNA supercoiling in bacteria"

In bacteria, DNA is maintained in a torsional stressed conformation due to the action of topoisomerase enzymes, resulting in DNA supercoiling in the form of twist and writhe. DNA supercoiling is largely known as a way to compact the chromosome, with variations in superhelicity reflecting rapid changes in environmental and metabolic conditions. By changing the physical state of DNA, it affects transcription in a global way, with a fraction of these promoters (such as those of ribosomal operons) being activated by negative supercoiling while others are repressed; yet a quantitative model of this global regulator is essentially missing. We will show some regulatory models developed in our lab, based on coarse-grained physical descriptions of the transcription process without any promoter-specific regulatory protein, and predicting significant contributions to the complex genome-wide response to superhelical variations.

Marc JOYEUX – LIPhy

"Associative and segregative phase separation cooperate in organizing the bacterial nucleoid"

The genomic DNA of bacteria occupies only a fraction of the cell called the nucleoid, although it is not bounded by any membrane and would occupy a volume hundreds of times larger than the cell in the absence of constraints. The two most important contributions to the compaction of the DNA coil are the cross-linking of the DNA by nucleoid proteins (like H-NS and StpA), which corresponds to an associative phase separation, and the demixing of DNA and other abundant globular macromolecules which do not bind to the DNA (like ribosomes), which corresponds to a segregative phase separation. This talk deals with the interplay of DNA-bridging proteins and globular macromolecular crowders, with the goal of determining the extent to which they collaborate in organizing the nucleoid. In order to answer this question, a coarse grained model was developed and its properties were investigated through Brownian dynamics simulations. These simulations reveal that the radius of gyration of the DNA coil decreases linearly with the effective volume ratio of globular crowders and the number of DNA bridges formed by nucleoid proteins in the whole range of physiological values. Moreover, simulations highlight the fact that the number of DNA bridges formed by nucleoid proteins depends crucially on their ability to self-associate (oligomerize). An explanation for this result is proposed in terms of the mean distance between DNA segments and the capacity of proteins to maintain DNA–bridging in spite of the thermal fluctuations of the DNA network. Finally, simulations indicate that non-associating proteins preserve a high mobility inside the nucleoid while contributing to its compaction, leading to a DNA/protein complex which looks like a liquid droplet. In contrast, self-associating proteins form a little deformable network which cross-links the DNA chain, with the consequence that the DNA/protein complex looks more like a gel.

Irina MIHALCESCU – LIPhy

"Quantitative study of low concentrations of DnaA *in Escherichia coli*, using the *uhp* expression system"

Chelli B[1], Van Melle-Gateau M[1], Lancelot L[1], Lazaro M[1], Boyat C[1], Coute Y[3], Ropers D[2], Geiselmann J[1,2], Mihalcescu I[1]

[1]UGA, CNRS, LIPHY, Grenoble; [2]UGA, Inria, Grenoble ; [3]UGA, CEA, INSERM, UA13 BGE, CNRS, CEA, Grenoble FR2048.

Precise regulation of DNA replication initiation is fundamental to bacterial cell cycle control. Here, we introduce a novel inducible activator system-the uhp expression system-to modulate DnaA production in *Escherichia coli* in response to external glucose- 6-phosphate (G6P), which, through deletion of *uhpT*, is designed here to function exclusively as an inducer.

By varying G6P concentrations in the growth medium, we achieve tunable control of DnaA levels under balanced growth conditions, ranging from 1- to 10-fold relative to wild-type cells. Upon removal of the G6P inducer, DnaA production ceases, and cells undergo a set number of additional divisions before division arrest, depending on their initial DnaA concentration, consistent with a reservoir depletion model. Single-cell analysis using a GFP reporter for uhp activity further reveals that, when initially induced to the average wild-type DnaA concentration, most cells divide three times before arrest, while even individual cells with smaller initial DnaA reservoirs-at the lower end of the distribution-are still able to complete at least one division before arresting.

These findings suggest that maintaining elevated steady-state levels of DnaA serves as a buMer, enabling cells to withstand fluctuations or interruptions in DnaA synthesis and ensuring continued cell cycle progression under variable conditions.

Corinne MERCIER – TIMC/TrEE

"Long bacterial cell" phenotype in the Long-Term Evolution Experiment (LTEE) on *Escherichia coli*, a selective advantage?"

Monge-Ruiz J[1, %, §], Antar HM[1,*,§], Kocak A[1], Efilé-Lukumba F[1], Rosenmann E[1], Hennebique A[1,2], Laurin D[4,5], Usson Y[1], Maurin M[1,2], Yamaryo-Botté Y[4], Botté C[4], Schneider D[1], Aldebert-Morin D[1], Schaack B[1,3], Hindré T[1], Mercier C[1]

[1]CNRS, UMR 5525, Vet'Agro Sup, Grenoble INP, TIMC, UGA, TrEE team; [2]Bacteriology - Hospital Hygiene Laboratory, Pharmacy Department, UGA; [3]CEA, CNRS, IBS, UGA; [4]INSERM U1209 & CNRS UMR 5309, Institute for Advanced Biosciences, UGA; [5]Etablissement Français du Sang, Département Scientifique Auvergne Rhône-Alpes; [§]equal contributions ; current addresses: [%] Unité de Bioénergétique et Ingénierie des Protéines, Team 09, UMR 7281 CNRS – Aix-Marseille University; [*]Department of Fundamental Microbiology, University of Lausanne, 1015 Lausanne, Switzerland.

Initiated by R.E. Lenski in February 1988, the Long-Term Evolution Experiment (LTEE) aims at studying the long-term evolution of *Escherichia coli* in a controlled environment to decipher the dynamics, the repeatability and the relationship between phenotypic and genotypic evolutionary changes: two strains (~ 2 µm long), respectively

REL606 (Ara-: unable to use arabinose as a carbon source) and REL607 (Ara+) were used to found 12 populations, 6 flasks inoculated with REL606 and 6 others, with REL607. The 12 flasks contained a minimal medium supplemented with only 25 mg/L glucose (DM25). Since 1988, one percent of each of these 12 flasks has been and is still transferred every 24 hours into a set of 12 clean flasks containing fresh medium. This technically simple experiment has generated, so far, more than 80,000 bacterial generations (80K) (https://the-ltee.org/) that have not been challenged by any antibiotic or immune response for more than 35 years. The 12 populations have rapidly adapted to their stringent environment by increasing their fitness, their growth rate and their volume.

Here, I will summarize the work we have performed on the characterization of two 50K-evolved clones from the respective populations Ara-3 and Ara-5. These clones have developed an exceptional length (up to 50 µm) at 50K generations of evolution. Mutations in their respective genome may explain the observed phenotype. Phenotypic changes affecting the divisome or the bacterial membranes are in agreement with a few of these mutations. This unusual phenotype questions the potential selective advantage for such unusually long bacterial cells within their respective populations in the specific conditions of the LTEE. This question is currently addressed by Elven Rosenmann in the laboratory (see the BactoGre 2025 related poster: “Long bacterial cell” phenotype in the Long-Term Evolution Experiment (LTEE) on *Escherichia coli*: focus on the bacterial populations)."

Audrey LE GOUELLEC – TIMC/TrEE

“From Metabolomics to Transcriptomics: Deciphering Polyamine Regulation Mechanisms in *P. aeruginosa* during Chronic CF Infection”

Mora V[1], Tidjani R[1], Moyne O[1], Jovien C[1], Maurin M[1,2], Bicout D[3], Toussaint B[1], Le Gouellec A[1]

[1]Team TrEE, Laboratoire TIMC UMR 5525, CNRS UGA Grenoble INP, CHU Grenoble Alpes

[2]Service de Bacteriologie CHU Grenoble Alpes

[3]Team EPSP, Laboratoire TIMC UMR 5525, CNRS UGA Grenoble INP, CHU Grenoble Alpes

Using a high-resolution untargeted metabolomics approach, we previously demonstrated that Pseudomonas aeruginosa (Pa) strains isolated from 32 adults with cystic fibrosis (CF) could be grouped into three distinct metabotypes, with strains within each metabotype displaying similar metabolic profiles. Among the discriminant metabolites, polyamines—particularly spermidine—emerged as key markers for metabotype classification. Our previous work revealed a correlation between increased bacterial production of these metabolites and higher cytotoxicity of Pa isolates.

The aim of this study is to identify the mechanisms by which Pa modulates its polyamine production during chronic infection in CF patients, and to determine how this modulation impacts the expression of its virulence factors.

Materials and Methods:

From a cohort of 66 isolates obtained from 32 patients, we selected 28 Pa strains and grouped them based on their polyamine production levels: high-producing (PH) and low-producing (PL) strains. We sequenced the genomes of all 28 strains and

performed transcriptomic analysis on 6 PH and 5 PL strains. Comparative genomics and transcriptomics were then used to investigate the mechanisms regulating polyamine production.

Results:

Phylogenomic analysis confirmed that the isolates from the same patient were evolutionarily related, consistent with clonal adaptation over time. Differential gene expression analysis between PH and PL strains confirmed our previous metabolomic findings, showing a correlation between increased expression of genes involved in polyamine biosynthesis and upregulation of Pa virulence genes. Multiple distinct mechanisms appear to underlie these observed phenotypes.

Discussion and Conclusion:

Using an integrative approach combining metabolomics, transcriptomics, and genomics, we identified how Pa can modulate its polyamine production and how this modulation may influence its virulence and contribute to the pathophysiology of chronic infection.

Eva GENDRON – TIMC/TrEE

"Dynamics of bacterial populations and their antibiotic resistance profiles in hospital wastewater from an intensive care unit"

Gendron E[1,2,3*], Schmidt V[3*], Hennebique A[2,3,4*], Terreaux-Masson C[5], Kurtz N[5], Charbonnier J[5], Maurin M[3,4], Mercier C[3], Landelle C[3,5], Buelow E[3]

[1]Université Claude Bernard de Lyon 1; [2]UFR de Pharmacie/UGA; [3]UGA, CNRS, UMR 5525, VetAgro Sup, Grenoble INP, CHU Grenoble Alpes, TIMC, Grenoble; [4]Laboratoire de Bactériologie, Institut de Biologie et de Pathologie, Centre Hospitalier Universitaire Grenoble Alpes, Grenoble; [5]Service d'Hygiène Hospitalière, Pôle de Santé Publique, Centre Hospitalier Universitaire Grenoble Alpes, Grenoble. * Equal contributions.

Antimicrobial resistance is a major public health threat, especially in hospital environments where antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) can emerge and spread rapidly. In this study, we describe the temporal dynamics of ARB, specifically carbapenemase-producing Enterobacterales (CPE), which are resistant to last-resort antibiotics, in hospital wastewater (WW) and WW biofilms (WWBs). The samples were collected monthly from a WW outlet joining the adult intensive care unit of the Grenoble Alps University Hospital over a period of 17 months (January 2023 to May 2024). The samples underwent conventional culturing methods associated with antibiotic susceptibility testing and phenotypic screening for carbapenemase producers, in accordance with the French national guidelines.

We observed shifts in the CPE population over time. From January to April 2023, *Citrobacter* sp. carrying the OXA-48 carbapenemase predominated, with *Serratia marcescens* carrying mainly VIM carbapenemase detected in minor proportions. Between May and December 2023, both species were detected in similar frequencies, while starting from January 2024, *S. marcescens* harboring VIM became the predominant isolated CPE.

Subsequently, whole genome sequencing was employed to compare the genomes of the isolated strains and to track their temporal evolution with a focus on

plasmids that carried the carbapenamases. Importantly, the CPE isolated from WW will be compared to CPE that were isolated from patients during the entire study period.

This study will provide valuable insights into the spread of ARGs in hospital environments and will highlight the importance of monitoring them to better understand the ARGs' dynamics and prevent their dissemination."

Aurélien TAUZIN – TIMC/MaGe

"Estimating bacterial mutation rates with simulation-based methods (ABC)"

The mutation rate is a key parameter of evolution as it defines the speed at which genetic diversity is generated de novo. It is defined as the number of mutation occurring per base pair per cell division (equal to generation for asexual organisms). A traditional approach to estimate mutation rate for bacteria is the fluctuation test, which consists in growing bacteria in a non-selective medium for then plating them in a selective environment in order to reveal the number of mutants (survivors) carrying a resistance to that stress. A mathematical model is then fitted on these experimental data to infer mutation rate. However, model makes restrictive assumptions regarding the effect of the mutations on the fitness (no effect) and the growth conditions (no death in the culture) which probably do not represent reality.

The objective of my work is to develop a new simulation-based computational method for the inference of mutation rate from experimental data (fluctuation tests), taking into account non-standard demographics (death) and fitness effect of the mutation. My approach relies on Approximate Bayesian Computations: it consists in using a simulator combined with MCMC algorithms (Metropolis-Hastings) to find the parameters which best explain the experimental data.

We tested it on two types of tasks:

- estimation of 1 parameter : mutation rate, when other parameters (death rate and fitness effect) are known

- simultaneous estimation of 2 parameters : mutation rate and fitness effect or mutation rate and death rate.

As today, this method already provides encouraging results. It competes well with the reference tools (rSalvador, which uses the Ma-Sandri-Sarkar maximum likelihood estimator, and Flan's probability generating function) in simple scenarios where these tools work. Due to its simulation-based nature, it can also generalize to more complex scenarios where these reference methods do not work.

Catherine LAROSE – IGE

"Microbial communities in glacier environments: Life below freezing"

Microorganisms colonize remote glacial ecosystems through atmospheric transport and deposition. These organisms generally arrive from other terrestrial habitats and must undergo selective or adaptive processes if they are to successfully colonize these frozen environments. Over short-time scales (hours to seasons), microbial communities exhibit dynamic metabolic responses to environmental triggers such as

water availability, light-dark cycles, nutrient pulses and community interactions in the snowpack. Once the snow is transformed to ice, we can use this archive to retrace the biosphere's evolution and response to environmental change over evolutionary time scales (centuries to millennia). Here, we will present results from a series of field and laboratory experiments that explore microbial activity and adaptation and present future research directions on tracing evolutionary processes using ice cores.

Sophie ABBY – TIMC/TrEE

"Dynamic quinone repertoire accompanied the diversification of energy metabolism in Pseudomonadota"

It is currently unclear how Pseudomonadota, a phylum that originated around the time of the Great Oxidation Event, became one of the most abundant and diverse bacterial phyla on Earth, with metabolically versatile members colonizing a wide range of environments with different O_2 concentrations. Here, we address this question by studying isoprenoid quinones, which are central components of energy metabolism covering a wide range of redox potentials. We demonstrate that a dynamic repertoire of quinone biosynthetic pathways accompanied the diversification of Pseudomonadota. The low potential menaquinone (MK) was lost in an ancestor of Pseudomonadota while the high potential ubiquinone (UQ) emerged. We show that the O_2-dependent and O_2-independent UQ pathways were both present in the last common ancestor of Pseudomonadota, and transmitted vertically. The O_2-independent pathway has a conserved genetic organization and displays signs of positive regulation by the master regulator "fumarate and nitrate reductase" (FNR), suggesting a conserved role for UQ in anaerobiosis across Pseudomonadota. The O_2-independent pathway was lost in some lineages but maintained in others, where it favoured a secondary reacquisition of low potential quinones (MK or rhodoquinone), which promoted diversification towards aerobic facultative and anaerobic metabolisms. Our results support that the ecological success of Pseudomonadota is linked to the acquisition of the largest known repertoire of quinones, which allowed adaptation to oxic niches as O_2 levels increased on Earth, and subsequent diversification into anoxic or O_2-fluctuating environments.

Reference: https://doi.org/10.1093/ismejo/wrae253

Timothée SALZAT-HERVOUETTE - TIMC/TrEE

"Investigating the evolution of phototrophy in Pseudomonadota"

Phototrophy is an ancient bacterial metabolism that likely originated over 3 billion years ago, prior to the rise of atmospheric oxygen provoked by oxygenic photosynthesis of Cyanobacteria. Phototrophy consists in the use of light for cellular energy production. This energy metabolism confers significant adaptive advantages to bacterial species in certain environments, yet it is sparsely distributed across several lineages of the bacterial tree of life. In the phylum Pseudomonadota (formerly Proteobacteria, also referred to as 'purple bacteria'), phototrophy is also sparsely distributed across various clades, yet it was proposed in the 1980s by Carl Woese that the ancestor of Pseudomonadota was a phototroph. Thus the question of the evolutionary origins and transmission of phototrophy across Pseudomonadota still

stands. The genetic potential for phototrophy is encoded by the photosynthetic gene cluster (PGC), a set of ~40 genes that colocalize in the genome and that gathers the genes required to produce photosynthetic pigments, as well as the reaction centers that are part of the light-harvesting antennae. Previous work by others found that the PGC could be found on plasmids, and a recent study suggested that the spread of phototrophy across the family Rhodobacteraceae was facilitated by horizontal gene transfer (HGT) of plasmids containing the PGC. The goal of the present study is to determine the respective contribution of vertical and lateral transmission of phototrophy in Pseudomonadota.To this end, an annotation tool based on the MacSyFinder program was designed for the annotation of PGC in bacterial genomes. More than 250 PGC were detected in 19,777 complete genomes of Pseudomonadota, which enabled subsequent phylogenomic analyses: genome context analyses and phylogenetic reconciliations between the PGC tree and the species tree. Overall, this study investigates the evolutionary history of phototrophy in Pseudomonadota and sets the grounds for a comparative genomic approach to investigate the metabolic factors underlying the sparse distribution of phototrophy across Pseudomonadota.

Eugenio CINQUEMANI – INRIA Center and LIPhy

“Characterization and control of microbial consortia on an automated mini-bioreactor platform”

Driven by both fundamental biological questions and potential biotechnological applications, the investigation of microbial consortia has become a focal point of research at the crossroads of microbiology, metabolic engineering, and control theory.

In this talk I will present our work on a synthetic consortium of two *E. coli* strains. A first, wild-type strain growing on glucose secretes acetate that at high concentrations impairs growth. A second strain modified to preferentially grow on acetate scavenges the latter from the culture. Investigation of coexistence conditions in chemostat experiments contributes to understanding analogous interaction patterns found in nature. Application of the consortium to the biosynthesis of molecules of interest (e.g., proteins or metabolites) has the potential to outperform single species in the production task.

I will illustrate our advances on the mathematical analysis and experimental characterization of the consortium, as well as perspectives on the real-time control of microbial consortia, in relation with a mini-bioreactor platform that we have developed for the automated monitoring and control of microbial cultures."

Jochen FICK – Institut Néel

“Optical trapping and swimming analysis of *Pseudomonas* bacteria”

The study of the bacteria swimming behavior or their interaction with other bacteria or cells requires an efficient and flexible tool for bacteria manipulation. Optical tweezers have been shown to be perfectly adapted for this task. Here we will present optical trapping of different species of the *Pseudomonas* genus using our optical fiber tweezers with dedicated structured optical fibers. Contactless trapping at low intensities was realized with 3D printed Fresnel lens fibers. Moreover, specific

swimming features and the behavior of trapped bacteria of the investigated species are compared applying different numerical methods.

Résumés des posters

1 - Delphine ALDEBERT – TIMC/TrEE

« Plateforme Technologique pour l'Imagerie du Vivant (PlaTIV) »

Camponova P, Fertin A, Aldebert D

UGA, CNRS, UMR 5525, CHU Grenoble Alpes, VetAgro Sup, Grenoble INP, TIMC, 38000 Grenoble

PlaTIV (Plateforme Technologique pour l'Imagerie du Vivant) est une infrastructure située sur le site Santé de Grenoble, au sein de l'unité TIMC. Labellisée IBISA et FBI, et membre de la fédération grenobloise ISdV (Imagerie des Sciences du Vivant), PlaTIV restructure ses activités pour mieux accompagner ses utilisateurs actuels et futurs. Elle offre un large éventail de compétences et d'équipements en microscopie, en traitement d'image et en cytométrie en flux, notamment dans un laboratoire de niveau de sécurité L2 adapté à la manipulation de microorganismes pathogènes.

L'expertise de la plateforme couvre :

- la microscopie à fluorescence automatisée,
- la cytométrie appliquée à l'étude des microorganismes (dénombrement, invasion, prolifération, interactions hôte-pathogène),
- le criblage de molécules anti-infectieuses.
- l'imagerie cellulaire et tissulaire en microscopie confocale (sur tissus fixés ou cellules vivantes/fixées),
- la vidéomicroscopie,
- la microscopie à tomographie optique cohérente,
- la microscopie conventionnelle (immunohistochimie, immunocytochimie)
- le développement de protocoles de traitement d'image.

Les travaux menés avec la plateforme illustrent la diversité de ses applications : suivi morphologique d'*E. coli* dans le cadre de l'expérience LTEE (Long-Term Evolution Experiment) via vidéomicroscopie, microscopie à fluorescence automatisée et microscopie confocale[1,2] ; observation de la fusion de vésicules extracellulaires fluorescentes avec les bactéries *E. coli*[3] ; suivi de la production de *Chlamydia*, bactéries intracellulaires, par microscopie automatisée[4] ; étude de la survie de *Francisella* dans des amibes par microscopie confocale[5] ; quantification de chitine sur des modèles fongiques[6] ; cribage de molécules anti-parasitaires[7].

PlaTIV adapte son expertise aux besoins des chercheurs et reste un partenaire clé pour le développement de projets en imagerie. Retrouvez toutes les informations pratiques et techniques sur notre site : https://www.timc.fr/plateformes-ressources

Références : 1- Hammam Antar: M2 report, 2019, M2 Immunology, Microbiology, Infectious Diseases (IMID). Encadrante C. Mercier ; 2 - Arif Kocak : M2 report, 2024, M2 IMID Encadrante C. Mercier ; 3 - Extracellular Vesicles from 50,000 Generation Clones of the *Escherichia coli* Long-Term Evolution Experiment : https://pmc.ncbi.nlm.nih.gov/articles/PMC9737989/; 4 - Mediation of Interleukin-23 and Tumor Necrosis Factor– Driven Reactive Arthritis by Chlamydia- Infected Macrophages in SKG Mice : https://acrjournals-onlinelibrary-wiley-com.sid2nomade-

1.grenet.fr/doi/full/10.1002/art.416531; 5 - Amoebae can promote the survival of *Francisella* species in the aquatic environment : https://pubmed.ncbi.nlm.nih.gov/33538648/; 6 - Easy-to-use imaging-cytometry assay to analyze chitin patterns in yeasts : https://pubmed.ncbi.nlm.nih.gov/38945044/; 7 - High-content imaging assay to evaluate *Toxoplasma gondii* infection and proliferation: A multiparametric assay to screen new compounds : https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0201678

2 - Delphine DEBARRE – LIPhy

« Controlling physico-chemical properties of solid/liquid interfaces to study surface colonization by motile bacteria »

Bacteria are able to attach to and explore solid/liquid interfaces under a wide range of chemical and mechanical conditions. Starting from individual bacteria that move and divide on a surface, microcolonies with time evolve into biofilms exhibiting increased resistance to antibiotics and mechanical removal, a major issue for human health.

We have developed a range of methods to control physico-chemical properties of solid interfaces and integrate them with microfluidic flow control. We use these methods to study how interface properties influence the efficiency of surface colonization and the structure of the early forming biofilms in P. aeruginosa, an opportunistic pathogen causing nosocomial infections. These properties include surface chemistry, surface charges, roughness, or rigidity. I will illustrate the insight that can be gained in the understanding of surface colonization on the case of surface exploration, which is shown to strongly affect early biofilms morphology.

3 - Marie COUTURIER – CERMAV

« A glycosaminoglycan degrading Polysaccharide Utilization Locus in the oral microbiota species *Segatella oris* »

Cousin A, Awad Y, Boustany R-J, Friedel M, Drouillard S, Touvrey-Loiodice M, Buon L, Helbert W, Vivès R, Couturier M

The human gut microbiota has been explored for the last two decades and studies have uncovered its major role in the degradation of complex glycans from the diet as well as the host tissues [1]. Strikingly, no similar investigation has been carried out on the human oral cavity microbiota, which is increasingly recognized has an important parameter in gastrointestinal health [2]. *Prevotellaceae* are an important and insufficiently investigated group of bacteria in both the gut and the oral cavity microbiota [3]. As other mucosal surfaces in the human host, the oral cavity is particularly rich in glycosaminoglycans which could provide an abundant carbon source for the inhabiting microorganisms. Hence, we identified a glycosaminoglycandegrading enzymatic systems in the oral microbiota species *Segatella oris*. We produced and characterized the corresponding enzymes, focusing more particularly our efforts on the characterization of the first *Prevotellaceae* endosulfatase. We show here that the oral microbiota species *Segatella oris* encodes a functional enzymatic system for the degradation of glycosaminoglycans. These results are a first step in the awaited exploration of the functional capabilities of the human oral microbiota.

References: [1] Warden et al, doi: 10.1038/s41579-022-00712-1 ; [2] Baker et al, doi: 10.1038/s41579-023-00963-6 ; [3] Tett et al, doi: 10.1038/s41579-021-00559-y

4 – Quentin FERNANDEZ DE GRADO – TIMC/MaGe

« What Selective Forces Act on the Order of Metabolic Genes in Operons? »

Bacterial genomes are organized in operons, grouping multiple genes that are expressed in similar biological conditions. We study the order of genes within operons, a question that has received little attention. The specific question we ask is whether two genes catalyzing two successive reactions of a metabolic pathway are encoded in the same order within their operon than they appear within the pathway. A pioneer study has shown 15 years ago that the answer is yes for more than half of such gene pairs, and suggested a mechanism leading to a selection pressure for coding genes in this order. On the opposite, in this work we are looking for selection pressures which could explain why a significant amount of gene pairs are encoded in the invert genetic order.

For all metabolic operons of *E. coli*, we computed several features which could directly or indirectly reflect the benefit of the invert genetic order, such as essentiality of each gene, fitness defect associated with knockout or over-expression of each gene, average expression level of the operon, potential toxicity of the intermediate metabolite between the two reactions, and conservation of the genes in their order in other organisms.

We found that fitness defect caused by knockout of the downstream gene within the pathway is significantly associated with the inverse gene order (in which this downstream gene is encoded first in the operon), suggesting that this inverse order arises from a selection pressure for strong and robust expression of the downstream gene. We postulate that in some pathways, this selection pressure could be caused by the toxicity of the intermediate metabolite, leading to a benefit in stronger and more robust expression of the gene which consumes the metabolite than the gene which produces it.

5 – Morgane ROGER-MARGUERITAT – TIMC/TrEE

« Quinone cross-feeding between gut bacteria linked to widespread occurrence of partial menaquinone biosynthesis pathways »

Roger-Margueritat M, Chobert S-C, Abby S, Pierrel F

The gut microbiota integrity is directly correlated to the host's health [1] and a way to maintain the diversity on its community is to promote the exchange of compounds between donor and recipient bacteria, this phenomenon called cross-feeding [2]. By this way, the discovery of new exchanges could be really useful to keep us healthy. Moreover, the potential exchange of quinones, hydrophobic key molecules in the respiratory chains in the whole tree of life, have been suggested but never characterized [3].

First, the bioinformatic annotation with HMM profiles of the menaquinone (MK) synthesis pathway, the predominant quinone in bacteria [4], has been performed on the UHGG dataset [5]. This reveals several cases of partial MK pathways where the

remaining proteins match with the possibility of precursors exchanges in the medium. Furthermore, phylogenetic analyses have been undertaken to study the transmission of the pathway and pointed to the loss of a part of the pathway instead of the horizontal transfer to gain this truncated pathway. To go further, experimental validations have been realized, validating partial MK pathway functionality and the use of precursors provided by other bacteria, and even by the host, has been confirmed in co-cultures. Structural analysis have been performed and revealed a crucial amino acid controlling the substrate preference. Finally, the whole quinone content in feces reveal a wide range of quinones and this new method is promising to confirm the quinone cross-feeding in vivo, this exchange may one day contribute to impact the dynamics of the gut microbiota.

References: [1] Daisley BA, Koenig D, Engelbrecht K, Doney L, Hards K, Al KF, et al. Emerging connections between gut microbiome bioenergetics and chronic metabolic diseases. Cell Rep 2021;37:110087. https://doi.org/10.1016/j.celrep.2021.110087. [2] Culp EJ, Goodman AL. Cross-feeding in the gut microbiome: Ecology and mechanisms. Cell Host & Microbe 2023;31:485–99. https://doi.org/10.1016/j.chom.2023.03.016. [3] Franza T, Gaudu P. Quinones: More Than Electron Shuttles.Research in Microbiology 2022:103953. https://doi.org/10.1016/j.resmic.2022.103953. [4] Bentley R, Meganathan R. Biosynthesis of vitamin K (menaquinone) in bacteria. Microbiol Rev 1982;46:40. [5] Almaida A, Nayfach S, Boland M, Strozzi F, Beracochea M, Shi Zj, et al. A unified catalog of 204,938 reference genomes from the human gut microbiome. Nature Biotechnology 2021;39. https://doi.org/10.1038/s41587-020-0603-3.

6 – Elven ROSENMANN – TIMC/TrEE

« Long bacterial cell phenotype in the Long-Term Evolution Experiment (LTEE) on *Escherichia coli*: focus on the bacterial populations »

Initiated by R.E. Lenski in February 1988, the Long-Term Evolution Experiment (LTEE) aims at studying the long-term evolution of *Escherichia coli* in a controlled environment to decipher the dynamics, the repeatability and the relationship between phenotypic and genotypic evolutionary changes: two strains (~ 2 µm long), respectively REL606 (Ara-: unable to use arabinose as a carbon source) and REL607 (Ara+) were used to found 12 populations, 6 flasks inoculated with REL606 and 6 others, with REL607. The 12 flasks contained a minimal medium supplemented with only 25 mg/L glucose (DM25). Since 1988, one percent of each of these 12 flasks has been and is still transferred every 24 hours into a set of 12 clean flasks containing fresh medium. This technically simple experiment has generated, so far, more than 80,000 bacterial generations (80K) (https://the-ltee.org/) that have not been challenged by any antibiotic or immune response for more than 35 years. The 12 populations have rapidly adapted to their stringent environment by increasing their fitness, their growth rate and their volume.

We have identified and characterized two 50K-evolved clones from the respective populations Ara-3 and Ara-5. These clones have developed an exceptional length (up to 50 µm) at 50K generations of evolution. Changes in bacterial length are usually related to the disruption of biological processes such as chromosome replication, cell division, cell elongation, cell wall assembly, and/or growth rate. We have identified, in the genome of these clones, mutations that may explain the observed phenotype and we have validated experimentally that, in contrast to the DNA

replication, which seems to be undisturbed, formation of the divisome and that of membranes are affected.

We are now focusing on the respective Ara-3 and Ara-5 populations to investigate whether the same phenotype can be identified in the evolved populations and importantly, whether mutations identified in the clones are also found in the metagenomes. These studies may allow answering whether this peculiar “long bacterial cell” phenotype constitutes, in the LTEE conditions of culture, a selective advantage that may lead to polyploidy.

7 – Béatrice SCHAACK – TIMC/CEA

« Bacterial versus host extracellular vesicles: critical elements of host-microbiota interplay »

Schaack B[1,2], Mercier C[2], Laurin D[3]

UGA [1]CEA, CNRS, Institut de Biologie Structurale ; [2]CNRS UMR 5525, Laboratoire TIMC TrEE ;[3]INSERM U1209, CNRS UMR 5309, Ets Français du Sang, IAB

Extracellular vesicles (EVs) are key players in inter- and intra-kingdom communications, particularly within animal holobionts - including humans. We addressed the question of exchanges between the intestinal microbiota and the host via EVs.

First, we analyzed the presence of bacterial EVs (BEVs) in healthy donors, in the absence of barrier disruption (on therapeutic blood products), in search of markers enriched in enterobacterial EVs. Next, we produced and used fluorescent Escherichia coli EVs to monitor their fusion with blood mononuclear cells. Finally, we followed their diffusion from the mouse intestine into the circulating blood to the organs.

Our study shows that BEVs are critical and highly active elements of cellular communication between the host and the microbiota.