MICROBIOLOGY DAY

5th edition

28TH OF MAY 2024

DOMAINE DU HAUT-CARRÉ

BOOKLET

STEERING COMMITTEE

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SPECIAL THANKS TO

Natacha Janiszewski, creator of the logo of this event

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MICROBIOLOGY DAY PROGRAM

8.30 am **Welcome**

9 am-9.10 am **Introduction**
Alexia Damour

9.10-9.30 am **Presentation of the Microbio-NA network**
Frédéric Bringaud

9.30-10.15 am - Physiology of pathogenic microorganisms
Chairman: Derrick Robinson

9.30 am: **Perrine HERVÉ**
Lipid droplets in the ancestral model organism *Trypanosoma brucei*

9.45 am: **Bachir BOUREIMA ABDOU**
Circularization, transcription and transfer capacity of integrative and conjugative elements of *Mycoplasma hominis*, human genital pathogen

10.00 am: **Yorick DAHAN:**
A role for fibril in *Spiroplasma* helical shape: from membrane curvature to morphological resilience

10.15 am-10.45 am - Coffee break

10.45 am-Noon - Environmental microbiology and technological aspects
Chairman: Thierry Noël

10.45 am: **Pierre-Alain MARON – Invited speaker**
La microbiologie des sols au service des productions agricoles

11.30 am: **Audrey BEAUSSART**
Microalgae at the heart of environmental and biotechnological concerns

11.45 am: **HOGAN Patrick**
Improved CRISPR-base editor tools for genome edition in *Mycoplasma bovis*: application to surface proteins

Noon-1 pm - La microbiologie dans l'industrie
Chairwoman: Sandrine Claus

12.00 pm: **Fatima M'ZALI:** Aquitaine Microbiologie
Aquitaine Microbiologie in the era of One-Health

12.20 pm: **Michaël TREILLES:** Qualyse
Qualyse : un laboratoire au service de la surveillance microbiologique du territoire. Exemple du diagnostic et de la surveillance en santé animale

12.40 pm: **Hubert DEBREYNE:** Novaptech
Development of aptasensors for antibiotic monitoring

1 pm-2 pm – Lunch

2 pm-3.15 pm - Host-pathogen interaction
Chairman: Charles Bodet

2.00 pm: **Nolwenn JOUVENET – Invited speaker**
Induction et évasion de la réponse interféron par les flavivirus

2.45 pm: **Chloé TORRES**
GCN2 kinase as a key factor to modulate viral replication

3.00 am: **Léa IBARLOSA**
Amyloid signaling in antiphage defense

3. 15 pm – 4.30 pm – Poster session & coffee break

4.30 pm-5.15 pm - Antimicrobial therapies and resistance
Chairwoman: Sabine Pereyre

4.30 pm: **Anne-Laure GUENIN**
Acinetobacter spp. "One-Health" strain collection: construction and characterization

4.45 pm: **Farras Daffa IMTIYAZ**
Adjuvant-loaded lipid nanoparticles to increase the susceptibility of *Klebsiella pneumoniae* to colistin

5.00 am: **Fabien JARRY**
Study of the *Achromobacter xylosoxidans* type VI secretion system and its clinical implication

5.15-5.25 pm – Sonia BURREL
Presentation of the Société Française de Microbiologie (SFM)

5.25-5.30 pm – Awards and concluding remarks
Alexia Damour

Perrine HERVÉ
Microbiologie Fondamentale et Pathogénicité - MFP - UMR 5234 – CNRS

Lipid droplets in the ancestral model organism *Trypanosoma brucei*

Perrine Hervé[1], Corinne Blancard[2], Bénédicte Salin[2], Yamaryo Yoshiki-Botté[3], Cyrille Botté[3], Frédéric Bringaud[1], Loïc Rivière[1]

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Lipid droplets (LD) are atypical organelles derived from the endoplasmic reticulum. These lipid-enriched vesicles are surrounded by a single leaflet of phospholipids crowned by proteins involved in LD biogenesis, maintenance and lipid metabolism, working in tightly-regulated processes to maintain cell lipid homeostasis. For more than a century, the importance of these organelles has been largely underestimated, thought as passive lipid inclusion bodies. However, in the past two decades, studies have shown their key functions in metabolic and immunological pathways. In protozoan parasites, LD represent an even more recent but growing interest. In Plasmodium sp., the agent of malaria, LD are involved in parasite survival inside red blood cells, participating in the detoxification of toxic by-products resulting from hemoglobin consumption. In Leishmania parasites, which cause leishmaniasis, LD are the site of arachidonic acid and prostaglandin production and may help parasites to survive under oxydative stress caused by environmental changes or antiparasitic drugs. In African trypanosomes, extracellular parasites responsible for deadly diseases in Humans (Sleeping sickness) and animals (Nagana, Dourine, Surra), the roles of LD remain to be understood. We have identified putative orthologs of several proteins involved in LD dynamics in Humans or yeasts. Using functional genomics and -omics approaches (CRISPR/CAS9, lipidomics…) in the model organism Trypanosoma brucei, we aim to unravel the functions of LD through characterization of TbPat, an LD-bound patatin-like phospholipase with an expression pattern tightly controlled by this pathogen.

Bachir BOUREIMA ABDOU
Microbiologie Fondamentale et Pathogénicité - MFP - UMR 5234 - CNRS

Circularization, transcription and transfer capacity of integrative and conjugative elements of *Mycoplasma hominis*, human genital pathogen

Bachir Boureima Abdou[1], Alicia Silvant[1,2], Chloé Le Roy[1], Jennifer Guiraud3, Léa Bientz[1,2], Véronique Dubois[1,2], Cécile Bébéar[1,2,3] and Sabine Pereyre[1,2,3]

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Integrative and conjugative elements (ICEs) are modular mobile genetic elements that can disseminate through excision, circularisation, and transfer. In Mycoplasma hominis (Mho), a human urogenital pathogen, 45% of isolates harbor integrative and conjugative elements (ICEs) integrated in the chromosome. These ICEs, of 27-30 kbp, harbor 25-30 CDSs, are present in one or several copies in the genome. In this study, our primary aim was to assess the ICE circularization capacities under different environmental conditions and investigate the transcription of the ICE CDSs. The second part of our study focused on functional investigations of the ICE through mating experiments.
Using qPCR and RT-qPCR experiments, we assessed the circularization and transcription of the Mho 4788 strain ICE (ICEHo-4788) under different conditions: exponential/stationary growth phase, exposure to mitomycin C, a DNA-damaging agent, to -80°C cold shock stress, and growth in HeLa cell culture. Functional studies involved mating experiments between donor strains carrying the ICE and recipient strains under both axenic and cell culture conditions.
Upon axenic growth, a circularization peak occurred at 12h of culture, with a 9.4-fold increase of circular forms. Exposure to mitomycin C and to cold-shock stress resulted in 3.3- and 3.0-fold rise in circular forms, respectively. In cell culture conditions, maximum circularization was observed at 72h and 7 days post-infection, with 10- and 23-fold increases, respectively. Regarding transcription, RT-qPCR experiments showed that all ICEHo-4788 CDSs were transcribed. Moreover, all RT-PCRs targeting intergenic region produced amplicons, suggesting that ICEHo-4788 is transcribed as a single polycistronic mRNA. No transcriptional variations were observed in ICE under mitomycin C exposure and cold shock stress. However, significant increases in ICE CDSs transcription were observed in cell culture conditions at 7 days post-infection with 5- to 23-fold increases. As for the functional study, no transfer of the ICE was achieved. However, we observed “Mycoplasma chromosomal transfer”, wherein the recipient strain transferred several parts of its chromosome to the donor strain, thus creating mosaic genomes.
This study emphasizes the influence of environmental conditions on the circularization dynamics and transcription of ICEHo-4788. Additionally, it marks the first observation of genetic material exchange in M. hominis.

Yorick DAHAN
Biologie du Fruit et Pathologie - BFP - UMR 1332 - INRAE

A role for fibril in *Spiroplasma* helical shape from membrane curvature to morphological resilience

Dahan Yorick[1], Lambert Bastien[1,2], Vilquin Alexandre[3,4], Bouttier Maxime[5], Dubrana Marie-Pierre[1], Decossas Marion[5], Lambert Olivier[4], Baret Jean-Christophe[2], Martin Nicolas[2], Béven Laure[1]

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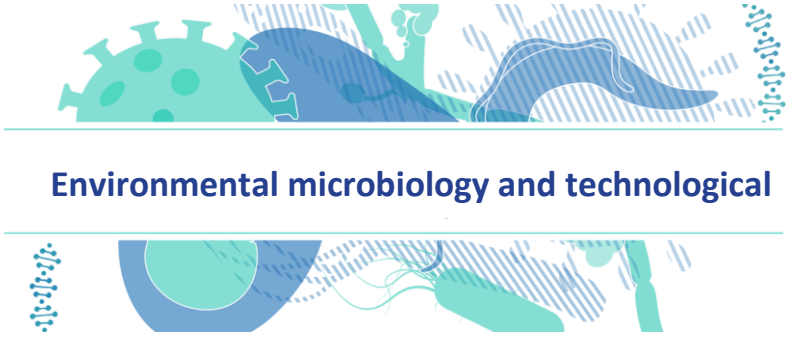
[4.]University Bordeaux, CNRS, LOMA, UMR 5798, F-33400 Talence, France
[5.]University Bordeaux, CNRS, CBMN, UMR 5248, Bordeaux INP, Pessac, France

In *Spiroplasma*, the helical shape and movement are orchestrated by an internal cytoskeleton primarily composed of two proteins: fibril (Fib) and SMreB. The kinking movement of *Spiroplasma* involves the inversion of helical chirality throughout the cell body. Fibril, exclusive to *Spiroplasma*, forms a ribbon that regularly interacts with the inner leaflet of the plasma membrane. SMreB proteins are bacterial actin-like proteins that polymerize *in vitro*, similarly to other bacterial or archeal MreB proteins, but their organization within the cell remains unclear. Depending on the species, spiroplasmas possess a variable number of SMreB gene isoforms. Solely, SMreB proteins have the ability to induce membrane curvature and promote cell helicity. Similarly, the fibril alone possesses this capability. However, the precise roles of SMreB isoforms and fibril in helicity remain somewhat elusive.
In our study, phylogenetic analysis reveals a gene gain of fibril in the Citri and Apis clades during evolution, prompting inquiries into its selective advantage for *Spiroplasma*. Comparison of fibril and SMreB expression levels, assessed through proteomics, coupled with statistical analysis of shape and motility parameters across 11 species differing in their fibril and SMreB content using video-microscopy, alongside intracellular cytoskeleton organization analysis in three species via cryo-electron microscopy, sheds light on the impact of fibril presence in the cytoskeleton. Significantly, fibril facilitates the preservation of a consistent helix pitch, which varies among species based on genetic background and SMreB content. Consequently, we posit a pivotal role for fibril in morphological resilience, enabling *Spiroplasma* to regain a helical shape, subsequently to the deformation due to the kink propagation. The presence of fibril could thus confer a selective advantage during *Spiroplasma*'s colonization of the host.



Alain Pierre MARON – Invited speaker
La microbiologie des sols au service des productions agricoles

Audrey BEAUSSART
Chimie et Biologie des Membranes et des Nano-objets - CBMN - UMR 5248 - CNRS - Bx INP – BSA

Microalgae at the heart of environmental and biotechnological concerns

N. Lesniewska[1], J.F.L. Duval[1], B. Sohm[1], C. Caillet[1], A. Razafitianamaharavo[1], I. Bihannic[1], S. El-Kirat-Chatel[2], A. Beaussart[2]

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Green microalgae have been the subject of growing interest both from fundamental and industrial points of view over the past years. As a representative of oil-accumulating cells, microalgae are considered as a promising sustainable resource for biofuel production capable of replacing fossil fuel. Their high yield in macronutrients is also beneficial to the sectors of food supplements and feeds, nutraceuticals, cosmetics, or fertilizers. From an environmental perspective, microalgae adsorb and accumulate various toxic compounds, which make them potential substrates in bioremediation processes of wastewater and polluted ecosystems. Being basic elements of the food chains in aquatic media, they are also key players in contaminant transfer through trophic levels.
In this study, we address the physico-chemical properties of the surface, including rigidity, electrostatics, and hydrophobicity, of the green algae *Chorella vulgaris* at various scales and for different environmental conditions. We show the importance of the growth phase on the cell-wall characteristics and how we can modulate the properties of the microalgae by varying the composition of their surrounding solution.
We then decipher how the algal surface properties influence their interaction with nanoparticles (NPs), and assess the molecular mechanisms behind NPs toxicity in multi-stress context. The originality of the approach relies on a combination of tools and methodologies from microbiology, physico-chemistry and biophysics that allow to identify and establish links between toxicity mechanisms measured at different scales. In particular, *via* the use of atomic force microscopy, still under-used to

answer ecotoxicological questions, interactions between cells and NPs are quantified from their first seconds of contact and their effects are visualized with a nanoscale spatial resolution. Using the autofluorescence of the microalgae and the fluorescence of the chosen NPs, confocal microscopy also allows to follow the kinetics of NPs adsorption and how the latter varies with pH modifications. Finally, we show the consequences of longer NPs exposure times (up to several hours) in terms of algal viability and physiological changes, such as pigment content.
Altogether, our results bring new light on the physical chemistry of complex systems and open promising perspectives to control and optimize conditions that would favor large-scale exploitation of the algal bioresource.

Patrick HOGAN
Biologie du Fruit et Pathologie - BFP - UMR 1332 – INRAE

Improved CRISPR-base editor tools for genome edition in *Mycoplasma bovis*: Application to surface proteins

Patrick Hogan[1], Fabien Rideau[1], Thomas Ipoutcha[1], Laure Beven[1], Pascal Sirand-Pugnet[1]

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Among the minimal bacteria belonging to the genus *Mycoplasma*, several of them are recognized pathogens for a wide diversity of animals. *Mycoplasma bovis* is one of the most significant species infecting dairy and fattening cows, causing mastitis and pneumonia, respectively. Attempts to control the disease have led to mass culling and costs of hundreds of millions in treatments and compensation worldwide, emphasizing the need for improved vaccines.
Within the RAMbo-V consortium (Rational Approach to a *Mycoplasma bovis* Vaccine), we believe that advances in genome engineering tools provide a unique opportunity to pave the way towards producing a vaccine strain of M. bovis expressing at its surface a specific set of conserved antigenic structures.
Advances in CRISPR-based tools, such as SpyCas9 recognizing PAM variants and base editors, have allowed for an increase in potential uses. These technologies paired with an algorithm predicting the complete array of potential targets within a given CDS library of *M. bovis* have allowed for a more in-depth look at the possibilities of these technologies and the feasibility of gene knockouts.
Here we report the use of multiple tools in *M. bovis* that led to the knockout of genes of interest such as the vsp (variable surface proteins) locus. VSPs are abundant and phase-variable components of the *M. bovis* membrane, which may participate in surface crowding and masking of stably expressed proteins, in addition to their putative biological functions. Targeting of the Xer1 tyrosine recombinase that permits spontaneous non-coordinate phase-variable expression results in the generation of phase-locked mutants.
The development of genome engineering tools will permit targeting virulence factors and optimized antigenic presentation. Such tools will be useful to build a vaccine chassis that would allow presentation of selected epitopes at the cell surface, leading to an improved immune response.

La microbiologie dans l'industrie

Fatima M'ZALI – Aquitaine Microbiologie

Aquitaine Microbiologie in the era of One-Health

Michaël TREILLES - Qualyse

Qualyse : un laboratoire au service de la surveillance microbiologique du territoire. Exemple du diagnostic et de la surveillance en santé animale

Hubert DEBREYNE – Novaptech

Development of aptasensors for antibiotic monitoring

Nolwenn JOUVENET – Invited speaker
Signalisation antivirale – Institut Pasteur Paris

Induction et évasion de la réponse interféron par les flavivirus

Chloé TORRES
Microbiologie Fondamentale et Pathogénicité - MFP - UMR 5234 – CNRS

GCN2 kinase as a key factor to modulate viral replication

Chloé Torres[1], Floriane Lagadec[1], Patricia Recordon-Pinson[1,2], Marie-Line Andreola[1,2], Mathieu Métifiot[1]

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GCN2 is a human protein kinase involved in cellular stress response. First known to control global translation upon amino-acid deprivation, GCN2 has lately been implicated in response to infections by pathogens, including RNA viruses. Indeed, our lab showed that GCN2 is activated during HIV-1 infection, interacts with and phosphorylates the integrase leading to a decrease in viral replication. Targeting the interaction of HIV-1 integrase with its partners is actively pursued to develop novel antiviral strategies for patients facing therapeutic failures due to resistance mutations. More recently, we showed that GCN2 is down-regulated during SARS-CoV-2 infection, and that it interacts with the nucleocapsid protein of the virus. Thus, targeting GCN2 and its protein-protein interactions may lead to the discovery of a general antiviral mechanism. In this context, we developed an in vitro assay based on the AlphaScreen technology to monitor the integrase-GCN2 interaction. We screened libraries of FDA-approved drugs (n=133) and natural extracts (n=420) and identified 19 chemical modulators of the interaction (5 stimulators and 14 inhibitors). Complete characterization of these molecules and several derivatives is underway. Altogether, our results show that GCN2 may become a key target to fight RNA virus infections.

Léa IBARLOSA
Institut de Biochimie et Génétique Cellulaires - IBGC- UMR 5095 – CNRS

Amyloid signaling in antiphage defense

Léa Ibarlosa, Sonia Dheur, Alexandra Granger-Farbos, Virginie Coustou, Sven J. Saupe

Regulated cell death (RCD) pathways play a central role in immune defense mechanisms in animals, plants, fungi and bacteria. RCD represents a form of altruistic cellular suicide, which limits the spreading of pathogens by hindering their replication, thereby promoting organismal survival in multicellular life forms and providing an advantage at the population level in single-celled organisms. In fungi, specific RCD pathways rely on the transmission of an amyloid fold from a receptor protein (generally a Nod-like receptor) to a cell death inducing effector protein (generally HeLo-Like domain membrane targeting protein). In its general principle, amyloid signaling is akin to prion propagation.
We now find that amyloid signaling also occurs in prokaryotes and is involved in antiphage defense. We have identified an operon we term Bab/Agp present in E. coli and related enterobacteria which confers resistance to various bacteriophages including the T5 phage. This operon codes for two proteins, Agp, a large multidomain receptor protein (comprising TPR-repeats, a GreA-related RNA polymerase-binding domain, and a PIN nuclease domain) and, Bab, a small protein with a predicted membrane targeting BELL domain (bacterial HeLo-like). Both proteins share an amyloid forming motif (BASS11 for bacterial amyloid signaling sequence) located at the N-terminus of Agp and at the C-terminus of Bab. Upon infection with the T5 phage, the Bab/Agp operon confers resistance by inducing a rapid cell-death reaction known as Abi response (for abortive infection) before phage replication can be completed. We will show that the Abi response relies on an amyloid signaling process by which the amyloid forming region of the Agp protein signals activation of the downstream Bab protein which then functions as a membrane-targeting cell death inducing effector.

Anne-Laure GUENIN
Anti-Infectieux : supports moléculaires des résistances et innovations thérapeutiques - RESINFIT - UMR 1092 – INSERM

Acinetobacter spp. "One-Health" strain collection: construction and characterization

Anne-Laure Guenin[1], Marie-Sarah Cayette[1], Jean-Luc Zonderland[2], Michaël Treilles[3], Elodie Couvé-Deacon[1,4] et Sandra Da Ré[1]

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[2]Departmental Analysis and Research Laboratory of Dordogne, Coulounieix-Chamiers, France
[3]Qualyse Laboratory, Niort, France,
[4]CHU Limoges, Bacteriology-Virology-Hygiene Laboratory, Limoges, France

Introduction and objectives: The *Acinetobacter* genus groups Gram-negative coccobacilli, including *Acinetobacter baumannii* (Ab), a multi-resistant opportunistic pathogen responsible for infections in humans and animals, and also found in the environment. Ab is considered by the WHO as one of the 6 priority pathogens for research into antibiotic resistance. It is a naturally competent bacterium with a plastic genome that enables it to acquire genetic elements associated with its multidrug resistance, such as resistance integrons (RIs). Although Ab is considered an environmental bacterium, little is known about its ecological niche. The aim of this project is to set up a "One-Health" collection of *Acinetobacter* spp. strains in order to identify potential Ab reservoirs and to determine whether there are links between Ab and non-*baumannii* strains isolated from humans, animals and the environment.

Material and methods: Human strains were isolated from patients at Limoges and Bordeaux university hospitals. Animal strains were isolated from pet and farm animals. Environmental strains come from the clinical environment and environmental samples (waters, soil with different level of anthropization). For the latter, strains were isolated on selective CHROMagar Acinetobacter medium and species were validated by MALDI-TOF mass spectrometry. Strains were first characterized for resistance (antibiogram), competence and integron presence (triplex PCR).

Results, discussion and conclusion:
To date, our Acinetobacter collection comprises 111 clinical strains, 84 animal strains and 158 environmental strains. Further animal and environmental samples are currently being collected. Of the 208 strains studied, 4.3% have RI (class 1 or class 2)

and 34% of the 89 strains tested are naturally transformable. Preliminary analysis suggest that Ab is a species mainly found in humans, whereas A. lwoffii seems to be more commonly found in animals (equidae more specifically). In terms of environmental samples, more than half of the samples tested positive for *Acinetobacter* with *A. johnsonii* found mainly in water and *A. bohemicus* in soil.
To identify possible links between *Acinetobacter* spp. strains from different environments, we will sequence the genome of a selection of strains from each environment (long-read NGS sequencing).

Farras Daffa IMTIYAZ
Pharmacology of Antimicrobial Agents and antibioResistance - PHAR2 - U1070 - INSERM

Adjuvant-loaded lipid nanoparticles to increase the susceptibility of *Klebsiella pneumoniae* to colistin

F.D. Imtiyaz [1], C. Faivre [2], J.M. Buyck [1], S. Marchand [1, 3], T. Henry [4], N. Anton [5], M. Collot [2], F. Tewes [1]

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[5] INSERM UMR 1260, Regenerative Nanomedicine (RNM), CRBS, Université de Strasbourg - Strasbourg (France)

The mobilized colistin resistance (mcr) gene has complicated the use of colistin against *Klebsiella pneumoniae* infection. Terpene alcohols and fatty acids are known as potential colistin adjuvant, but their direct clinical use is difficult due to their high lipophilicity. Here, we tested the efficacy of lipid nanoparticles (LNPs) loaded separately with four adjuvants as a colistin potentiator in vitro and in vivo against K. pneumoniae. Four LNPs were screened as colistin adjuvants against five clinical strains and one mcr-1 mutant of *K. pneumoniae* having different colistin susceptibility using checkerboard and time-kill curve (TKC) experiments. The most potent colistin-LNP combination was studied for its effects on bacterial membranes using propidium iodide (PI) uptake and scanning electron microscopy (SEM). Subsequently, the in vivo efficacy of the combination was also tested on the Galleria larval infection model. (E, E)-farnesol LNPs reduced colistin MIC better than the other three LNPs against all tested isolates in a strain and concentration-dependent manner. Interestingly, the most striking colistin boost was up to 1024-time MIC reductions against the mcr1 mutant (MIC = 4-8 mg/L). TKC against that strain showed that (E, E)-farnesol LNPs at 60 mg/L + as low as 0.13 mg/L colistin eradicated viable bacteria with no regrowth at the later time point, but their single administrations were not. PI results showed that the combination enhanced fluorescence faster than its single administration. SEM also confirmed surface damage of bacteria in the presence of the combination. Survival of larvae infected with 106 CFU/larvae was 10% and 30% at day 7 post-infection after being treated with colistin and the combination, respectively. Overall, our in vitro and in vivo results showed that this combination has the potency to control resistant K. pneumoniae infection.

Fabien JARRY
Anti-Infectieux : supports moléculaires des résistances et innovations thérapeutiques - RESINFIT - UMR 1092 – INSERM

Study of the *Achromobacter xylosoxidans* type VI secretion system and its clinical implication

F Jarry[1], A Patry[1], C Bordi[2], E Durand[3] and F Garnier[1]

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[3]Mediterranean Institute of Microbiology (IMM), Aix-Marseille University, UMR 7283, Marseilles

Achromobacter xylosoxidans is a Gram-negative bacillus that has been described over the last ten years as an emerging pathogen, particularly in cystic fibrosis. The urge to combat antimicrobial resistance is driving the search for new therapeutic alternatives. One promising lead is to block virulence factors. The type VI secretion system (T6SS) is a highly conserved bacterial nanomachine, found in over 25% of Gram-negative bacteria, which releases effector molecules for bacterial competition and infection of eukaryotic cells. A study of the genome of an *A. xylosoxidans* environmental A8 strain revealed the existence of two putative TAX-1 and TAX-2 clusters encoding T6SS. A collection of 61 *Achromobacter* strains was assembled and screened for the presence of TAX-1 and TAX-2. The results of the screening demonstrated that 57 strains (93.5%) harbored TAX-1, while only 4 (6.5%) harbored TAX-2. Having demonstrated that TAX-1 expression depends on the strain, its origin, and its environment, the aim of this research was to investigate TAX-2 expression by quantifying the transcripts of the hcp2 gene belonging to TAX-2 in the four strains that harbored it, in comparison with A8.
A specific PCR screening coupled with Sanger sequencing procedure was employed to demonstrate that all strains did indeed possess hcp2.
After developing a method for quantifying transcripts by RT-qPCR, we demonstrated that the strains exhibited low levels of hcp2 expression, comparable to or lower than that observed in A8.

Posters

List of posters

1	ANGER	Robin	Structure of a heteropolymeric type IV pilus from a Gram positive bacterium
2	ABADIE	Maïder	Impact of the microbial community and mycotoxin production during grain storage in soil-dug silos
3	AUFFRAY	Julie	Development of a new antibody formulation in tablet form and its application to the innovative treatment of recurrent vaginal candidiasis
4	AUTIN	Michel	Interplay between HIV-1 integration and cellular transcription
5	BARAQUIN	Marine	Sustainable production of waxes through biotechnology
6	BERLUREAU	Julien	An atypical F-like atpase is critical to the function of the antibody cleavage system MIB-MIP
7	BHATTACHARYA	Arunima	Context-dependent inhibition of bacterial translation by the odilorhabdin class of peptide antibiotics
8	BOHUON	Emma	Getting close insights into deep-sea vents microbial ecosystems
9	CALMELS	Christina	Study of structure-fonction of Bet, Foamy virus protein and MB21D2, its cellular partner
10	DUFLOT	Gaëlle	Development of an in vivo selection system of ligand-dependant arrest peptides in E. coli
11	GILLET	Léo	Study of the resistome and virulome of H. pylori by NGS approach using DNA capture technology
12	GOURIN	Claire	Study of pUL77 and pUL93, two partners of the HCMV terminase complex
13	JEHANNE	Quentin	First isolation of *Campylobacter ovis* in humans suffering from gastroenteritis.
14	JITTASEVI	Jitra-Marie	Deciphering genome transplantation mechanisms as a step towards understanding basic principles of life
15	KADOUCH	Mathilde	Implementation and application of a p53 prionization model decoupled from its inherent function
16	LAMBERT	Chloé	Characterization of an intriguing protein, BILBO3, in the flagellated pathogen *Trypanosoma brucei*
17	LAPAILLERIE	Delphine	Cell response to incoming retroviral genomes, role of the BRCA1/2 DNA repair pathway on the regulation of non-integrated and integrated viral DNAs
18	LE HENAFF LE MARREC	Claire	Prévalence, organisation et répertoire des satellites de phages chez la bactérie lactique *Oenococcus oeni*
19	LE ROY	Chloé	In Vitro Selection and Characterization of Resistance to Josamycin and Pristinamycin in Mycoplasma genitalium
20	LOPEZ GARCIA	Fernanda	Intestine-on-chip as a model to study Candida yeast infections

21	MACHADO DA SILVEIRA	Juliane	Evolutionary engineering and mutagenesis as alternatives to improve S*pathaspora passalidarum* for second-generation ethanol production
22	MANDART	Marie	Structural and functional characterization of bacterial spirosomes
23	MENARD	Maëlenn	What is the influence of biocalcifying bacteria on the biofouling of metallic structures under cathodic protection in marine environments?
24	NAVARRO	Marine	Communication between *Fusarium* : role of miRNAs
25	OVIEDO HERNANDEZ	Florencia	Phage bacteria co-evolution in wine. The virulent Krappator 27 phage selects for unique genomic changes in the lactic acid bacterium *Oenococcus oeni*.
26	PARISSI	Vincent	Host chromatin invasion by retroviral genomes
27	ROUILLAN	Louis	Structural and functional study of the type VIIb secretion system (T7SSb) of *Bacillus subtilis* involved in bacterial competition
28	SCILLIA	Lisa	Targeting β-lactamase expression with lipid antisense oligonucleotides
29	SIMANKOV	Nikolay	ARSENAL: Antimicrobial ReSistance prEdictioN using a mAchine Learning approach
30	TOUYA	Aurélie	Modulation of enniatin yield in *Fusarium* avenaceum: a focus on oxidative stress
31	TYMCHENKO	Anastasiia	Adenovirus spreading and restriction factors in primary bronchial epithelia
32	VACHER	Louis	Étude en temps réel de la localisation de l'ADN viral du Prototype Foamy Virus

1. Structure of a heteropolymeric type IV pilus from a Gram positive bacterium

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Type IV filaments (T4F) represent a ubiquitous superfamily of nanomachines found in Bacteria and Archaea, comprised mainly of filamentous polymers of type IV pilins. These filaments play pivotal roles in adhesion, motility (both swimming and twitching), DNA uptake, bacterial community formation, and protein secretion, making them significant virulence factors in various bacterial pathogens.
Recent advancements in cryo-electron microscopy (cryoEM) have unveiled conserved helical architectures of T4F, revealing intriguing "melted" segments within the α1N portion of pilins during filament polymerization.
In contrast to previous studies focusing on Gram-negative bacteria, our research expands this understanding by investigating T4F architecture in phylogenetically distant Gram-positive species. We present a 3.7 Å resolution cryo-EM structure of *Streptococcus sanguinis* heteropolymeric T4F, providing a comprehensive atomic model encompassing all pilins (major and minor). Our findings highlight universal features of bacterial T4F and have widespread implications in understanding T4F biology.

2. Impact of the microbial community and mycotoxin production during grain storage in soil-dug silos

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Cereal storage in underground silos was used from the Neolithic period until now in many populations across the world. For thousands of years, soil-dug silos were an essential commodity for life and survival of human populations. It also represented an important economic feature in some communities, as found in France during the Middle Age. The SilArchaeoBio project is based on an experimental archaeology approach, trying to recreate pre-industrial pits in two sites, in the Perpignan area. The project is looking at the preservation of three different cereals (ancient wheat, barley and einkorn) in underground silos, dug with similar means than pre-industrial populations (when possible). After a variable duration of storage, the grain properties were analysed: germinative capacity, grain quality and food-safety assessment. Furthermore, a thorough monitoring of the storage conditions is applied. Several probes were used to record the temperature, humidity and CO2 concentration in several locations inside the silos, across a several-months experiment.
A two-phase sampling (spring and summer) provided enough material to analyze more

parameters in the lab. A mycotoxins dosage (Aflatoxins and ochratoxins) was processed and the results were correlated with a quantification of microbial communities by quantitative PCR: ITS marker to estimate the fungi population and 16S marker to evaluate the bacteria. A precise composition of the grain microbiome will be assessed by metabarcoding, to hopefully establish a link between storage conditions (variations in temperature and humidity) and the prevalence of some microbial populations like mycotoxins producers.
In parallel, further experiments are processed in controlled environments, to simplify a complex system influenced by numerous parameters (forecast, soil properties, insects invasion). Microcosms are used to study the kinetics of the initial development of the grain microbiome. A simple experimental design allows to test microbial growth on three types of cereal, in a controlled temperature and humidity set-up. Mycotoxins titration and qPCR analysis will allow a better understanding of the early phase of grain colonisation by microbes.
Thanks to all the findings obtained both in the lab and in the underground silos, a stronger knowledge on fungal and bacterial populations equilibrium will allow a better grasp on grain storage and how we could use/revisit ancient preservation techniques to improve cereal storage, save energy and lower the impact of agriculture on climate change.

3. Development of a new antibody formulation in tablet form and its application to the innovative treatment of recurrent vaginal candidiasis

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Vulvovaginal candidiasis (VVC) is a fungal infection predominantly caused by the opportunistic pathogen species *C. albicans* (90%). VVC is a common infection that affects 75% of women at least once in their lifetime. However, in 8% of cases, the infection can become recurrent (RVVC) with at least four episodes per year. Current treatments for (R)VVC mainly rely on the use of azole antifungals, which are effective in treating isolated episodes but insufficient to prevent recurrences. In addition, the intensive use of azoles may contribute to the development of resistance, highlighting the necessity to develop therapeutic alternatives. Considering several arguments indicating that VVC may result from an immunopathological disorder characterized by a ""neutrophil anergy"" phenomenon, wich is responsible for an exacerbated inflammatory response, the first objective of this project was to develop biological tools to assess the effectiveness of passive immunization as an alternative treatment for RVVC. In this study, we selected and characterized monoclonal antibodies (mAbs) raised against two targets of the hypal infectious form of *C. albicans*: the toxin Candidalysin (Cdlys) and the cell wall protein Hyr1. The mAbs were then used in combination for passive immunization experiments in a mouse model of VVC with two objectives (1) modulate the host's inflammatory response and (2) stimulate the phagocytic efficiency of vaginal innate immune cells. Our results showed that neutralization of Candidalysin with anti-Cdlys mAb modulates the intensity of the inflammatory reaction in the vaginal

environment during VVC, and that, in parallel, opsonization of *C. albicans* using anti-Hyr1 mAb decreases the fungal burden at the vaginal epithelium.
Additionally, the second objective of this study was to explore the possibility of formulating these mAbs into tablet form. Tablets offer several advantages over the conventional injectable forms commonly used for therapeutic mAbs, including ease of administration, lower production cost, and high stability at room temperature. Importantly, tablets can enable the local delivery of biomolecules, especially via oral or vaginal routes (directly to the epithelium). However, the tableting of mAbs has not been extensively described in the literature. Considering the extreme fragility of mAbs, their structural integrity, and consequently, their biological activity, may be altered by the application of high mechanical stresses during tableting. By carefully selecting compression conditions (including formulation and process parameters), our findings indicate that compaction has no detrimental effect on the integrity of the mAbs. Specifically, we demonstrated that the binding properties of both mAbs remained fully intact after tableting. This observation was further reinforced by in vitro experiments, which demonstrated the preservation of the biological activity of both anti-Cdlys and anti-Hyr1 mAbs after tableting.

4. Interplay between HIV-1 integration and cellular transcription

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The host chromatin invasion by retrovirus relies on the stable integration of the viral genome into infected cell chromosomes. This integration is catalyzed by the intasome, a complex formed by the retroviral integrase (IN) enzyme and viral DNA. HIV-1 IN not only plays a role in the integration process but has additional functions, such as binding to genomic viral RNA and assisting in virion assembly. Furthermore, recent studies have reported that after integration, IN may remain associated with the integration site and that punctual mutations in the carboxyl-terminal domain known to bind RNA affect IN stability and viral gene transcription (Winans Shelby and Stephen P Goff, 2020). More recently, it has been shown that the binding of IN to TAR RNA induces structural changes in both IN and TAR, enhancing Tat binding and potentially optimizing the transactivation of viral DNA (Cecilia Rocchi et al., 2022).

Based on the interaction found between IN and TAR RNA, we speculated that the binding of TAR RNA to IN after the integration step may destabilize and dissociate the integration complex from the nucleosomal site. To investigate this hypothesis, we performed in vitro dissociation experiments of integration complexes reconstituted onto human nucleosomes using various RNA or DNA structures. RNA fragments were found to dissociate integration complexes, whereas DNA structures appear to be much less efficient. This dissociation was confirmed using Biolayer interferometry, on top of

showing that TAR RNA element would be the most effective for dissociating integration complex by binding IN. In addition, this dissociation was shown to be specific for HIV-1 since PFV wasn't affected by RNA. Furthermore, biochemical characterization of the composition of nucleoprotein complexes released after RNA-induced dissociation revealed that TAR RNA binding to IN leads to the dissociation of IN.LEDGF/p75 complex from the nucleosomal integrated product, as what would be expected in infected cells. Taken together, our data suggest a new functional interplay between HIV-1 integration and transcription which requires to be precisely coordinated to enable virus's optimal invasion of chromatin.

Correlation analysis between integration, IN fate and transcription during cellular infection using Tat-dependent or Tat-independent viral vector are under progress to confirm our hypothesis. Selection of specific IN/TAR chemical modulators using HTRF and molecular docking has also been initiated to develop new tools for depicting this mechanism and potentially producing the first antiviral agent acting at the early post-integration stage.

5. Sustainable production of waxes through biotechnology

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Waxes are esters of fatty acid and fatty alcohol, produced by some bacterial species when they face a depletion of an element, like nitrogen. They are accumulated in the form of granules, which serve as carbon and energy storage. The accumulated carbon is assimilated when the deficient element becomes available again. Waxes have a variety of applications, including cosmetics, paints and inks, lubricants and food additives. The objective of this study is to optimize wax ester production from waste feedstock by using the bacterium Marinobacter nauticus. This bacterium is capable of accumulating wax ester when nitrogen is limiting or when it grows as a biofilm. Several carbon sources, such as used frying oils and whey, were used to assess the growth and the production of wax ester by M. nauticus. The stages of wax ester formation and degradation were studied by fluorescence microscopy using a specific dye staining apolar lipids. These analyses allowed us to determine the number of wax granules produced per cell as well as the morphological characteristics of the bacterium during wax ester production in nitrogen starvation. The production of wax by M. nauticus could open up a new way of producing natural wax to reduce the quantity of wax produced by petrochemical plants while reusing waste products.

6. An atypical F-like ATPase is critical to the function of the antibody cleavage system MIB-MIP

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Biologie du Fruit et Pathologie - BFP - UMR 1332 – INRAE

Mycoplasmas can cause chronic infections suggesting that they are able to evade their host's immune system. We have previously described and characterized a system called MIB-MIP, used by mycoplasmas to selectively capture and cleave antibodies. The system is found in the majority of animal-pathogenic mycoplasmas. Interestingly, a set of 7 genes encoding a putative atypical F1-like X0 ATPase is genetically associated with MIB-MIP, and is often found at the same locus forming an operon. This co-occurrence and conservation suggest that this ATPase could be involved in the antibody-cleavage process.
To investigate this hypothesis, we generated a set of mutant strains of *Mycoplasma mycoides* subsp. capri GM12. We either performed a clean deletion of the F1-like X0 ATPase loci without affecting the MIB-MIP part of the operon, or performed a single amino-acid substitution by replacing the putatively catalytic Lysine 152 of the Walker A domain of the beta-subunit. Both mutants were fully validated through genome sequencing and mass spectrometry. We then assessed the ability of the mutants to capture cleave immunoglobulins, and compared it to the wild type strain.
The analysis performed showed that the mutations introduced in the F1-like X0 ATPase locus had no significant effect on the global proteome of the bacteria and that the MIB-MIP loci was still expressed in similar amounts compared to the wild-type. However, a functional comparative study showed that the both the ΔF1-like X0 ATPase and K152A mutants were no longer able to process antibodies.
Our data confirm the hypothesized involvement of the atypical F1-like X0 ATPase in the MIB-MIP system, and demonstrate that ATP binding is an essential step of the process. However, the exact function of the ATPase is remains unknown.

7. Context-dependent inhibition of bacterial translation by the odilorhabdin class of peptide antibiotics

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Acides nucléiques : Régulations naturelles et artificielles -ARNA - U1212 - INSERM - UMR5320 – CNRS

Odilorhabdins (ODLs) represent a new class of antibacterial non-ribosomal peptides that bind to a unique site in the 30S ribosomal subunit. ODLs establish direct contacts with the anticodon loop of the A-site tRNA, leading to decreased translation accuracy at lower drug concentrations, preventing the transition of tRNA from the A to the P-site, and inhibiting translocation at high drug concentrations. Previous experiments have indicated that the binding of the drug stalls the ribosomes in a context-dependent manner, and hence we performed inverse-toeprinting coupled with next-generation sequencing (iTP-Seq), a profiling method that locates ribosomes on a library of mRNAs with codon resolution. We then measured the frequency of amino acid motifs of various lengths in samples treated with the ODL NOSO-502, and in untreated samples, and

calculated the fold change in frequency of each motif after antibiotic treatment. Interestingly, context-dependent stalling at Glu-Pro (EP), Asp-Pro (DP) and Pro-Pro (PP) motifs (at the E- and P-sites respectively) was observed for a concentration of NOSO-502 near its half-maximal inhibitory concentration (IC50), whereas no context-dependence was observed in the presence of a higher concentration of the drug. To validate these observations, we plan to conduct toeprinting assays on a series of four amino acid motifs spanning the -2, -1 (E), 0 (P) and +1 (A) sites, and eventually delineate the structural basis of the stalling using cryo-electron microscopy.

8. Getting close insights into deep-sea vents microbial ecosystems

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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. These ecosystems host a wide variety of microorganisms with unique capabilities, pushing the boundaries of life as well as extended applications in biotechnology (e.g. using extremophilic microorganisms). However, they 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 geo-microbial interactions within the DSVs. In the frame of my PhD project, I study these specific environments, and representatives of archaea living within them, at lab scale using microfluidics and millifluidics reactors under extreme conditions. These small-scale reactors 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).

9. Study of structure-fonction of Bet, Foamy virus protein and MB21D2, its cellular partner

Christina CALMELS, Elisa GUILLON, Sebastien FRIBOURG, Stéphane THORE, Vincent PARISSI, Paul LESBATS.

Microbiologie Fondamentale et Pathogénicité - MFP - UMR 5234 – CNRS

Foamy viruses are endemic retroviruses in animals, and can also infect humans by zoonotic transmission. They are classified as a distinct subfamily than Orthoretroviruses due to many peculiarities amongst which their non-pathogenicity, an integration in non-coding regions and a very large tropism. This makes Foamy viruses particularly interesting for gene therapy, and potentially oncolytic virotherapy. In order to optimize

these therapeutic applications, though, it is necessary to elucidate the molecular mechanisms underlying these characteristics and the relationship between host and spumaretroviruses.
In this context, this project aims at characterizing the Bet auxiliary protein, wich exact function and structure remain unknown. Therefore, this project focuses on the structural characterization of Bet via X ray crystallography.
To study its function during infection, we have first searched for Bet cellular partners and thus, we have identified protein MB21D2, of unknown structure and function. We have developed proteomic approaches to look for Bet and its cellular partner.
Structural and functional characterization of both these proteins will be exposed in this poster.

10. Development of an in vivo selection system of ligand-dependant arrest peptides in *E. coli*

Gaëlle DUFLOT, Mathilde LARTIGAU, Thibaud RENAULT, Axel INNIS

Acides nucléiques : Régulations naturelles et artificielles -ARNA - U1212 - INSERM - UMR5320 – CNRS

Arrest peptides are a type of peptides that can cause the ribosome to stall during their own translation. A subset of them, called ligand-dependant arrest peptides, trigger ribosome stalling upon binding of a small molecule. They act as sensors and play a role in modulating the expression of downstream genes in response to their ligand: at the transcription level by preventing Rho-dependant transcription termination or at the translation level by freeing sequestrated ribosome binding sites or RBS.
It is thus important to be able to characterize natural ligand-dependant arrest peptides, to improve our understanding of biological processes that are regulated by them, and also to identify novel arrest peptides because of their potential applications in biotechnologies, such as biosensors.
We developed an in vivo selection system that is based on two components: a plasmid encoding libraries of arrest peptides, and a genomic array of genes comprising selection and reporter genes. Our system enables the detection of ribosome stalling depending on the presence of a ligand with positive and negative selection. We validated the negative and positive selection capabilities using model arrest peptides and mutants. We are now further testing the reporter modules and de novo selection of novel arrest peptides using random libraries.

11. Study of the resistome and virulome of H. pylori by NGS approach using DNA capture technology

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Background
The identification of *Helicobacter pylori* infection on gastric biopsies requires the use of PCR or bacterial culture. *H. pylori* culture is fastidious and may remain falsely negative

because of the fragility of the bacterium. The use of Next-Generation Sequencing (NGS) from human samples is one solution to directly have access to the resistome and virulome. Here, we describe the implementation of a new method to study directly from the gastric biopsy the bacterial resistome and virulome of *H. pylori*, based on the enrichment and sequencing of H. pylori DNA.
Methods. In total, 37 DNA samples from gastric biopsies from patients (mean age of 46.9 and sex ratio of 0.67) suffering from gastritis and non-ulcer dyspepsia were studied. All samples were positive for H. pylori using real-time PCR. Over 37 biopsies, 21 were positive by culture and 16 were negative. Agilent SureSelect XT DNA capture protocol was used and adapted on the Magnis NGS Library Prep System prior to sequencing. NGS sequencing was performed on the Illumina Iseq100 sequencer. RNA probes were used to target virulence (cagA and vacA), resistance (23S rDNA, 16S rDNA, gyrA, rpoB, pbp1, rdxA, frxA and fdxb) and molecular typing genes. These sequences were also obtained using Sanger sequencing from 21 DNAs of the *H. pylori* culture and PCR-positive biopsies in order to compare both methods.
Results. Mutations within the 23S rDNA sequence (3 A2142G,9 A2143G, 1 A2142C) associated with macrolide resistance and within the QRDR sequence gyrA (1 N87I, 4 N87K and 1 D91N) associated with levofloxacin resistance and mutation L525P in rpoB associated with rifabutine resistance were correctly identified. However, molecular identification of metronidazole resistance markers was unsuccessful. MLST phylogeny analyzes performed were concordant with those obtained on sequences obtained by traditional Sanger strategy. Among cagA-positive isolates, the gene was perfectly detected.
Conclusions. We showed here that DNA capture method allows to easily and precisely access *H. pylori* resistome and virulome directly from gastric biopsies. Such new method may still need to be applied to stools and paraffin-embedded samples.

12. Study of pUL77 and pUL93, two partners of the HCMV terminase complex

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Introduction and objectives:
Cytomegalovirus is the leading cause of congenital viral infection, responsible for high morbidity in transplant recipients. Letermovir is an antiviral agent targeting the CMVH encapsidation complex composed of the pUL56, pUL89 and pUL51 proteins. The complex's partner proteins, pUL77 and pUL93, have been shown to play an indispensable role in encapsidation. However, little is known about their precise functions and whether they may be linked to the mode of action of letermovir. The aim of this study is therefore to define their structure-function relationships.
Materials and methods:
The identification of conserved regions was carried out by alignment with ClustalW of 18 sequences of pUL77 and pUL93 homologues from different herpesviruses with strain AD169. The UL77 and UL93 genes of 5 reference CMVH strains, 30 strains naïve to any

letermovir treatment and 13 strains from patients treated with LTM were sequenced by SANGER or NGS. The results were complemented by 45 sequences from Genbank naïve strains. Amino acid mutations were listed in the form of two binary heatmaps. 3D modeling was performed using the I-TASSER and ALPHAFOLD servers. Mutations selected under letermovir were studied in recombinant virus.
Results, discussion and conclusion:
Four conserved domains were identified in pUL77 and three in pUL93. Two novel mutations were identified in each protein in letermovir-treated patients, suggesting a possible interaction of these with letermovir. Modeling of these two proteins revealed domains such as a coiled-coil motif in the first hundred amino acids of pUL77. Antiviral and replicative assays were carried out to determine the impact of these new mutations. A recombinant virus study is also planned to confirm the presence of putative NLSs described in the literature for each of the two proteins."

13. First isolation of *Campylobacter ovis* in humans suffering from gastroenteritis.

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Bordeaux Institute of Oncology - BRIC - U1312 – INSERM CHU de Bordeaux

The present study describes the first isolation in humans of a recently described species of *Campylobacter, Campylobacter ovis*, by two French independent laboratories in 2020 and 2022 from a man and a woman suffering from gastroenteritis. Biochemical, growth characteristics and electron microscopy for these two strains indicated that these isolates belong to Campylobacter genus. 16S rDNA and gyrA-based phylogeny, as well as Average Nucleotide Identity and in silico DNA-DNA Hybridization analyses revealed that both strains belong the *C. ovis* species. Both patient isolates possess a complete Cytolethal Distending Toxin (CDT) locus with cdtA, cdtB and cdtC features of CDT activity were demonstrated in vitro with Caco-2 intestinal epithelial cells. Our data suggest that these two isolates of C. ovis were associated with gastroenteritis in humans and induces major cytopathogenic effects in vitro. That novel species, *Campylobacter ovis*, is likely to be a novel human pathogen, with the source of foodborne infection could be the sheep. *Campylobacter* species which display toxicity features are a worldwide public health issue and it is crucial to identify which isolate could be an urgent threat to a patient. The description of that species within humans is as valuable for the medical field as it is for the microbiologist community.

14. Deciphering genome transplantation mechanisms as a step towards understanding basic principles of life

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Building a cell from the ground up would help identify the minimal set of elements necessary for a cell-like compartment to become a functional living entity. This requires an extensive understanding of the contribution of each component to the cell function. This project focuses on genetic information and its processing by a compartment capable of gene expression, using an original approach named whole genome transplantation (WGT). This technique consists in isolating a whole bacterial genome belonging to Species A (donor genome) and installing it in the cytoplasm of Species B (recipient cell), resulting in cells genotypically and phenotypically identical to Species A.
WGT is currently performed on Mollicutes, the simplest living forms capable of autonomous replication outside of a host, an ideal model for studying essential requirements for life. Understanding what defines the compatibility between the donor genome and recipient cell may lead to identifying key elements that enable booting up a living cell and to understanding the rules that regulate the interactions between genetic material and the compartment which expresses it. We hypothetize that the ability of the recipient transcription machinery to "interpret" the data encoded on the donor chromosome is essential for successful boot-up. Our approach consists in engineering a recipient cell, *Mycoplasma capricolum* (Mcap), to preload it with transcription factors belonging to a donor genome, *Mesoplasma florum* (Mflorum).
The coding sequences of the five subunits of the Mflorum RNA polymerase were cloned into plasmids along with their native promoters, individually or in combination. Transformation of Mcap suggests that the Mflorum genes can be expressed individually and in combination in Mcap and that their presence does not impact the recipient's survival. In the next phase, the Mcap cells expressing the Mflorum RNA Polymerase will be used as recipients for WGT assays and structural analysis of their transcription machinery will be performed.

15. Implementation and application of a p53 prionization model decoupled from its inherent function

Mathilde KADOUCH, Sabine CASTANO, Christelle MARCHAL, Christophe CULLIN

Chimie et Biologie des Membranes et des Nano-objets - CBMN - UMR 5248 - CNRS - Bx INP – BSA

The term ""prion"" originally refers to the infectious agent responsible for transmissible spongiform encephalopathies such as mad cow disease and Creutzfeldt–Jakob disease. Initially categorized as an ""unconventional virus"" (D.C. Gajdusek, Nobel 1976), it was later identified as a protein (S.B. Prusiner, Nobel 1997): the PrP or Prion Protein. PrP exists in two stable conformations: a soluble, non-pathogenic state and an aggregated, pathogenic state. Aggregated PrP can recruit soluble PrP, forming an autocatalytic loop that stabilizes the aggregated form and enhances its transmissibility. This model can be extended to other proteins, such as yeast prions (R.B. Wickner, 1994) and human

proteins implicated in neurodegenerative diseases.
Remarkably, the tumor suppressor protein p53 also displays prion-like features, forming amyloid aggregates capable of cell-to-cell transmission, as evidenced by recent studies (Ghosh et al., 2017). Given its potential role in cancer initiation and progression, reactivating p53 presents a promising therapeutic approach. To this end, we propose to develop a prionization model for p53 independent of its function. This model will enable us to investigate the impact of p53 loss-of-function mutations on its aggregation and to screen for synthetic p53 alleles that modulate the aggregation process, paving the way for novel therapeutic strategies."

16. Characterization of an intriguing protein, BILBO3, in the flagellated pathogen *Trypanosoma brucei*.

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Trypanosoma brucei (*T. brucei*) is a unicellular and flagellated parasite that causes human and animal African trypanosomiasis. Its flagellum exits the cell body through the flagellar pocket (FP). The FP is formed by an invagination of the plasma membrane at the base of the flagellum and is the unique site of endo and exocytosis. The FP is maintained enclosed around the flagellum by the flagellar pocket collar (FPC). The FPC is an essential cytoskeletal structure but its composition, structure and function are poorly known.
The team previously identified and characterized the first FPC proteins, BILBO1 and BILBO2. Their N-terminals domains (NTD) are similar in structure with several essential residues that are conserved. We have identified three others proteins with their NTD share 30% homology with the BILBO1-NTD. Among these proteins is BILBO3 for which no data have been yet published. The aim was to characterize BILBO3 by studying its localization, function and potential proteins partners. I showed that BILBO3 localizes at different cytoskeleton structures suggesting that it could play critical roles in the biogenesis of the FPC. Further using BioID, I identified several uncharacterized BILBO3 partners revealing higher complexity than expected in the components involved in FPC structure or function.

17. Cell response to incoming retroviral genomes, Role of the BRCA1/2 DNA repair pathway on the regulation of non-integrated and integrated viral DNAs

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Mobility of retroviral genomes triggers cellular responses that participate in the establishment of stable integration of the virus into the Host chromatin. Viral genome insertion requires the delivery of the integration complex (intasome) to the chromosomal insertion locus followed by the catalysis of integration and post-integrative events. These events include the integration complex disassembly, DNA

repair of the insertion site and viral gene transcription. We have previously shown that the DNA homologous repair (HR) processes can modulate both the HIV-1 pre-integrative and post-integrative stages (1–3).
We report here that nuclear foci of the HR RAD51 recombinase are triggered during the early phases of the retroviral infection by a mechanism dependent on the BRCA1/BRCA2 repair pathway. CHiP and imaging approaches indicate that RAD51 is loaded onto the incoming viral DNA as soon as it is synthesized during reverse transcription and before integration into chromatin. Inhibition of this process leads to a decrease in viral infectivity and integration associated with inhibition of reverse transcription and increased persistence of unintegrated viral DNA forms.
Our results thus reveal a new mechanism of cellular response to incoming retroviral genomes participating in regulating the early reverse transcription process, the fate of the different populations of viral DNA and possibly the establishment of persistent and/or latent HIV-1 virus reservoirs.

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ReactIN SIDACTION grants

18. Prévalence, organisation et répertoire des satellites de phages chez la bactérie lactique Oenococcus oeni

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La bactérie lactique *Oenococcus oeni* est responsable de la bioconversion de l'acide malique en acide lactique dans les vins. Cette étape, nommée fermentation malolactique, s'impose pour l'ensemble des vins rouges puisqu'elle confère souplesse, rondeur et stabilité microbiologique au produit final. Pour maîtriser la fermentation et obtenir des vins de qualité, il est donc essentiel d'identifier les facteurs susceptibles de limiter le développement de O. oeni au cours de la vinification. Les facteurs physico-chimiques du vin jouent un rôle essentiel et la combinaison de plusieurs paramètres (T°C, teneur en alcool et en composés polyphénoliques, pH …) affecte la biomasse bactérienne. Au sein de cet écosystème particulier, des facteurs biotiques conditionnent également le développement de *O. oeni*. Parmi eux, la présence de bactériophages antagonistes a été rapportée à toutes les étapes de la transformation du raisin en vin, et de nombreux siphophages purement lytiques [1] ou tempérés[2] actifs sur la bactérie *O. oeni* ont été caractérisés. L'essor du séquençage de génomes a confirmé la prévalence de la lysogénie chez O. oeni et 54% des souches contiennent 1, 2 ou 3 prophages actifs dans leur génome [3]. L'approche in-silico a également mis en évidence la présence de nombreux prophages vestigiaux [3]. Leur réexamen grâce à de nouveaux outils performants comme SatelliteFinder [4] permet aujourd'hui de les classer parmi les satellites de phages. Les interactions bactériophages-bactéries sont donc affectées par ces éléments qui exploitent les phages pour le transfert entre bactéries.
Notre étude a pour objectif d'identifier leur nombre, taille et diversité, et de présenter le

répertoire de gènes associés chez *O. oeni.* Une étude détaillée par qPCR de l'excision et du transfert du satellite I2 chez la souche lysogène *O. oeni* LAB6 sera également présentée.

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19. In Vitro Selection and Characterization of Resistance to Josamycin and Pristinamycin in *Mycoplasma genitalium*

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Macrolide resistance has emerged in *Mycoplasma genitalium*. Pristinamycin, a streptogramin combination, is part of the recommended third-line treatment according to the European guidelines. No data regarding mechanisms of pristinamycin resistance have been available to date. In *M. pneumoniae*, a phylogenetically close species, cross resistance to pristinamycin and josamycin was associated with mutations in the 23S rRNA gene at position 2062 (*Escherichia coli* numbering). We aimed to investigate the in vitro development of resistance in *M. genitalium* in the presence of subinhibitory concentrations of josamycin and pristinamycin.
Selection of resistant mutants was performed by serial passages of *M. genitalium* G37 reference strain in FRIIS medium containing subinhibitory concentrations of josamycin or pristinamycin. Resistant mutants were characterized by PCR amplification and Sanger sequencing of 23S rRNA, L4 and L22 ribosomal protein genes. For each resistant mutant, MICs of seven antibiotics were determined. Whole genome sequencing (WGS) was then performed on selected mutants using the Illumina technology.
A mutant selected in the presence of josamycin harbored an A2059G mutation in 23S rRNA. This mutant showed a strong increase in the MICs of erythromycin, azithromycin, josamycin, and clindamycin (16-32 µg/mL for all four antibiotics), but no changes in MICs of pristinamycin, doxycycline, and moxifloxacin (all three at 0.125 µg/mL).
Two mutants selected in the presence of pristinamycin harbored the mutation A2062C or A2062G. Both mutants showed a strong increase of the MICs of pristinamycin (8 and

2 µg/mL, respectively) and josamycin (32 and 16 µg/mL, respectively) and a slight 4 to 8-fold increase of erythromycin MIC. Azithromycin, clindamycin, doxycycline and moxifloxacin MICs remained unchanged. No mutations in L4 and L22 gene were detected in any mutants.
WGS of the three mutants confirmed the 23S rRNA mutations associated with antibiotic resistance and revealed 6 to 10 additional SNPs per mutant compared to the G37 reference strain sequence. These SNPs were located all around the genome and were not likely to be involved in resistance.
This study showed that resistant mutants can be selected in vitro in *M. genitalium* using josamycin and pristinamycin. These laboratory-derived mutants could be predictive for mutations observed in clinical strains.

20. Intestine-on-chip as a model to study Candida yeast infections

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Conventional in vitro biological models (cells in a Petri dish or Transwell inserts) fail to recapitulate the complex physio-biology of the human body. Alternatively, mouse models are now avoided not only because of ethical issues but also because of a lack of overlap between human and rodents (Cunningham, 2002). Organs-on-chips (OOCs) are an alternative to model organ functionality and recapitulate some of their physiological or pathological features in vitro (Huh et al., 2010). Even though the two-chamber commercial design of OOC is almost ideal to recapitulate the physiological conditions encountered in the intestine, its operational design intrinsically does not allow to observe real-time events under flow in culture compatible conditions. The overall objective of the project is to develop a new generation of OOCs in conditions that closely mimic the in vivo configuration, i.e. allowing the application of external mechanical cues (flow and stretching). The combination of a confocal microscopy module for high-resolution (but slow) fluorescence imaging with an Optical Coherence Tomography (OCT) module for lower (~µm) resolution but fast and label-free acquisition is envisioned. We aim to provide an in-depth investigation of the mechanisms underlying intestinal infection by *Candida* yeast with the perspective of identifying new routes for therapeutic treatments. The Intestine-on-chip consists of a microfluidic chip with 2 micro-channels separated by a central porous membrane, on either side of which epithelial cells and vascular endothelial cells will adhere, mimicking the interface of a vascularized human organ. Two lateral vacuum channels allowing the generation of mechanical stretching of the membrane will be included to mimic in vivo intestinal cells environment.

21. Evolutionary engineering and mutagenesis as alternatives to improve *Spathaspora passalidarum* for second-generation ethanol production

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Second-generation ethanol (2G ethanol) stands out in the search for sustainable alternatives in fuel by using lignocellulosic biomasses, such as sugarcane bagasse, which is largely industrial waste. However, 2G ethanol production's biggest challenge is the need to pretreat the biomass to solubilize sugars and reduce its recalcitrance. This stage is crucial but also releases inhibitory compounds, such as acetic acid (AA), that are extremely harmful to most yeasts, including *Spathaspora passalidarum*, which is a great fermenter for 2G ethanol production since it efficiently metabolizes xylose, the most abundant sugar in the resulting hydrolysate. To overcome this bottleneck in the process, it is possible to improve the yeast, making it more robust and resistant to the inhibitory compounds. Evolutionary engineering (EE) and mutagenesis have been proven effective, but there is a shortage of studies on their application for S. passalidarum. The objective of this study was to improve the ethanol production of the yeast S. passalidarum from hemicellulosic hydrolysate (HH) obtained by the acid pretreatment of sugarcane bagasse. Genetic improvement was performed by EE in combination with ultraviolet light (UV light) or ethyl methanesulfonate (EMS) mutagenesis. Five media containing HH (in g/L: 42 – 59 sugars, 0.86 – 2.30 AA) were used for three EE strategies: 1) 30 batches with increased HH concentration every 10 batches; after strategy 1: 2) 20 batches with the last previous medium; 3) 20 batches with increased HH concentration every 10 batches. Each batch was done at 30 ºC and 200 rpm for 12 h. From that, colonies were isolated, and the strains JY3103, JY3301, and JY5102 were tested for the improved fermentative potential of the HH3 medium with high cell density (20 g/L initial cell concentration, 30 ºC, 110 rpm, and 48 h), in comparison to the parental strain. The strain JY5102 stood out with an ethanol yield (YEtOH) and productivity (QEtOH), respectively, 11.19 ± 0.76 % and 15.64 ± 2.01 % higher than the parental strain. Moreover, the xylitol yield (YXylitol) for JY5102 was 24.23 ± 1.79 % less than the control. JY5102 was then used for UV and EMS mutagenesis assays until the survival rate was between 40 and 70 % each. Colonies were tested for growth and ethanol production with low cell density, and two strains (JY5102UV, JY5102EMS) were selected for HH fermentation experiments with high cell density in comparison to JY5102 and the parental strain. YEtOH of JY5102UV and JY5102EMS were higher than JY5102 and the parental strain, reaching 0.39 ± 0.00 and 0.42 ± 0.01 g/g, respectively. Similarly, QEtOH values were also improved, reaching 0.23 ± 0.01 and 0.21 ± 0.01 g/L h. YXylitol decreased by approximately 28.22 ± 4,61 % and 62.16 ± 4.17 % compared to the parental strain. The evolutionary engineering of the yeast S. passalidarum, combined with UV and EMS mutagenesis, resulted in the selection of improved strains with improved fermentative capabilities. It was noticeable that the yeast's adaptation to greater ethanol production from HH is possibly linked to the decrease in xylitol production.

22. Structural and functional characterization of bacterial spirosomes

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Acetaldehyde-alcohol dehydrogenases (AdhE) are key enzymes in bacterial metabolism and pathogenicity. They are also being investigated for their involvement in biofuel production. These enzymes are well conserved in all bacterial kingdoms, and are also found in more phylogenetically distant microorganisms such as green algae. During bacterial alcoholic fermentation under anaerobic conditions, AdhE catalyses a two-step reaction that takes place within its two distinct domains: the conversion of acetyl-coenzyme A to acetaldehyde by the N-terminal ALDH (acetaldehyde dehydrogenase) domain, and then the conversion of acetaldehyde to ethanol by the C-terminal ADH (alcohol dehydrogenase) domain. This reaction is associated with NAD+ regeneration, which is essential for glycolysis. The polymeric form of AdhE, called spirosomes, exists either in a compact conformation in the absence of substrate or cofactor, or in an extended form in the presence of cofactors (Fe2+, NAD and coenzyme A). In Escherichia coli, spirosomes are found in both conformations, whereas in Streptococcus pneumoniae, for example, spirosomes are constitutively present in the extended conformation. Previous results suggested that the extended conformation might be the active form of AdhE. The spirosome extension has been shown to be responsible for the channelling of the toxic acetaldehyde.
To explore the mechanisms that regulate the conformational switch between the extended and compact forms of AdhE spirosomes, we used cryo-electron microscopy (cryoEM) to determine the structure of constitutively extended spirosomes from S. pneumoniae. In parallel, we re-examined the structure of *E. coli* spirosomes in their extended state, as previously described by our group, and serendipitously noticed the presence of an extra density at the interface between adjacent ALDH and ADH domains, in some of the cryoEM maps. We then compared them in detail with those of *S. pneumoniae* spirosomes and observed structural differences that may be involved in the transition between compact and extended conformations. We used in vivo complementation assays to test mutations on bacterial growth under micro-aerobic conditions."

23. What is the influence of biocalcifying bacteria on the biofouling of metallic structures under cathodic protection in marine environments?

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When a metal surface is immersed in seawater, it is quickly colonized by microorganisms that cooperate to form a biofilm. This association of different microorganisms gives specific properties to biofilms, creating anchor points for other organisms (invertebrates, macroalgae, etc.). This process of colonization of an immersed substrate is known as biofouling. These biofouling deposits can also be associated with complex mineral deposits on the metal surface, such as calcareous deposits. Indeed, these deposits are formed when the metal is placed under cathodic protection to protect

it from corrosion. Cathodic protection leads to an alkalization of the cathode-seawater interface, shifting the calcocarbonate equilibrium, resulting in the precipitation of calcium carbonate and magnesium hydroxide, which form the calcareous deposit. Some bacteria, known as biocalcifying bacteria, are also known to promote the precipitation of calcium carbonate ($CaCO_3$).
Due to the presence of marine biocalcifying bacteria, the formation of this mixed mineral/biological fouling could be accelerated on structures protected cathodically, potentially leading to damage during their operation. To date, the understanding of the mechanisms leading to this mixed fouling are limited, so it is important to better understand them to avoid such problems.
To achieve this, various experiments are carried out in the laboratory. We produce calcareous deposits on carbon steel by cathodic polarization in marine environments in the presence of biocalcifying bacteria. This allows us to test different fouling formation conditions. We have thus demonstrated that biocalcifying bacteria have a visible impact on the formation of the deposit, particularly in its composition. Indeed, the deposits are composed of aragonite and brucite when formed from seawater alone. However, in the presence of different marine biocalcifying bacterial strains, its composition changes. In parallel, we conducted experiments under hypoosmotic conditions to approximate the conditions of estuarine waters where some ports are located. Our initial results show that the lower the salinity, the greater the production of $CaCO_3$ by marine bacteria."

24. Communication between Fusarium: role of miRNAs

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Mycologie et Sécurité Alimentaire - MycSA - UR 1264 – INRAE

Fusarium graminearum is the main agent of fusarium head blight (FHB) of small grain cereals and producer of mycotoxins resistant to agricultural practices and food processes. This fungal disease is caused by a complex of species of phytopathogenic fungi, including the genus *Fusarium*. Worldwide, FHB represents an important economic loss due to a loss of crop yield and a sanitary problem with the presence of mycotoxins dangerous for health. Recently, intra-microbiota interactions in plants have been shown to play a key role in infection and mycotoxin production. These interactions are orchestrated in part by microRNAs, which are small non-coding RNAs that induce post-transcriptional silencing of genes. These miRNAs are differentially expressed according to environmental conditions and interactions with other organisms present in the flora. Increasing evidence indicates that the molecular dialogue between Fusarium species during *Fusarium* head blight is essential for the outcome of the disease in the pathological system. The aim of this research is to investigate the role of fungal small RNAs in this dialogue. To achieve this, confrontation tests were set up with different species of Fusarium. Based on these tests, we performed transcriptomic analyses (RNAs and miRNAs) to identify the miRNAs produced during inter-species communication. Then, the transcriptomic data will be studied using an in-silico approach. This approach will enable us to catalog the miRNAs expressed during *Fusarium* interactions and to identify the putative targets of these miRNAs. Finally, these results will provide us with keys to the diagnosis and prevention of FHB and mycotoxin accumulation in the plant system.

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

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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 OE33PA1,2,3, 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 was 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.

26. Host chromatin invasion by retroviral genomes

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Microbiologie Fondamentale et Pathogénicité - MFP - UMR 5234 – CNRS

Integration of retroviral genome requires the association between the viral integration complex (intasome) and host chromatin involving multiple interfaces between the integrase (IN), the target DNA and the histone components of the nucleosome. These associations are regulated by cellular factors and the structure of the chromatin surrounding the targeted insertion site. Part of our project aims to identify these functional interfaces and to analyze the influence of cell factors regulating integration.
We characterized the IN-chromatin interactions by biochemical approaches using reconstituted chromatin and chromosomes spreads. This allowed us to highlight the intrinsic properties of IN to bind chromatin and its regulation by its cellular LEDGF/p75 cofactor. We have also shown the importance of both histone tails and the carboxy-terminal CTD domain of IN in this process and demonstrated that the neighboring nucleosomes modulate the functional binding of the intasome to the substrate nucleosome. The use of mutations targeting these interfaces confirmed that they participate in the efficiency of integration but also in the insertion site selection both in vitro and in infected cells. Altogether, these data suggest that the retroviral IN CTDs act

as sensors of the chromatin structure by scanning available histone and DNA interactions participating for the selection of optimal functional interfaces, and, thus for efficient genome invasion.
Based on these results, we have then developed biophysics approaches for monitoring the complexes formed by the retroviral intasome and the human nucleosome using BLItz and ALphaLISA. This work led us to quantify for the first time the interactions between these central partners of integration and to characterize the main parameters of association. On the basis of these interactions, we were finally able to establish a screening system for chemical compounds targeting this intasome-nucleosome complex. This screen allowed us to identify new drugs capable of inhibiting viral replication by targeting the histone tails, the nucleosome structure or the DNA/intasome interfaces. These new drugs compounds could constitute tools for dissecting further the integration process but also original therapeutic agents for antiviral or curative strategies.

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27. Structural and functional study of the type VIIb secretion system (T7SSb) of *Bacillus subtilis* involved in bacterial competition

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Microbiologie Fondamentale et Pathogénicité - MFP - UMR 5234 – CNRS

Bacteria live in densely populated, diverse environments where they compete with each other for nutrients, resources and space. Bacteria use various strategies to compete with other bacteria, including the secretion of toxins into competing bacteria. To do this, bacteria have evolved secretory systems to secrete effector proteins through their cell envelope into their extracellular environment or directly into eukaryotic or bacterial cells through their own cell envelope.
The type VII secretion system (T7SS) was initially discovered in mycobacteria because of its role in the virulence of Mycobacterium tuberculosis. In fact, the T7SS is absent in the live attenuated BCG vaccine strain, making it less virulent. The T7SS has also been found in other bacteria of the phylum Bacillota (Firmicutes), such as Bacillus subtilis and Staphylococcus aureus, and plays a key role in bacterial competition. Competition allows bacteria to regulate the composition of bacterial populations in biofilms and microbiota, especially during infection. However, there are many differences between the T7SS of mycobacteria and that of Bacillota, which is why they have been named type 7 ""a"" and ""b"" secretion systems, respectively. Many structural data are available for the mycobacterial T7SSa, but very few for the T7SSb.
Bacillus subtilis T7SSb is encoded by the yuk operon. The operon encodes membrane components, soluble cytosolic proteins and secreted toxins. These toxins are, for example, RNases that induce growth inhibition in target strains that do not have the corresponding antitoxin.
The aims of this project are to gain new insights into the structure and function of T7SSb in B. subtilis, including understanding how toxins are recruited, how bacterial toxins are

secreted through this system, the assembly and activation mechanisms of T7SS, and whether this system is contact-dependent.
A variety of methods will be used to achieve these goals, including fluorescence microscopy, bacterial competition assays, purification, cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET).
This research project aims to provide new structural information on T7SSb and shed light on its mechanism in bacterial competition. Bacillus subtilis T7SSb serves as a valuable model for understanding more complex T7SSb systems in pathogenic bacteria such as Staphylococcus aureus"

28. Targeting β-lactamase expression with lipid antisense oligonucleotides

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Extended-spectrum β-Lactamase (ESBL)-producing enterobacteria are classified by WHO as a priority for R&D. ESBL are enzymes that confer resistance against the most of β-lactam antibiotics including third-generation cephalosporin (3CG). CTX-M 15, the most prevalent ESBL in Europe, is mainly produced by Escherichia coli responsible of care-facility and community setting infections. New therapeutic strategies should be developed to prevent the lack of available antibiotic against multidrug-resistant bacteria in the future. For this, our project consists to the inhibition of the resistance gene by using antisense oligonucleotides (ASO). ASO will be used as adjuvants co-administrated with antibiotics to reduce resistance levels in bacteria. The objective is to inhibit the high-level expression of CTX-M-15 in E. coli by using modified ASO and to restore sensitivity of ceftriaxone (3CG). ASOs inhibit CTX-M-15 gene expression via base-pairing to the complementary strand of mRNA. This strategy allows targeting all genes, with high specificity and an easy design. Consequently, the aim is to determine the mechanism of action of LASO (lipid antisense oligonucleotide) and its interaction with the bacteria envelops and intracellular content. The specificity of the biological effect upon oligonucleotide sequence and chemistry will still have to be demonstrated. LASO will be optimized to provide a new therapeutic tool for fighting against drug resistance based on inhibition of gene."

29. ARSENAL: Antimicrobial ReSistance prEdictioN using a mAchine Learning approach

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Antimicrobial resistance (AMR) has emerged as a major public health concern due to the rapid proliferation of multidrug-resistant bacteria, posing significant challenges in the prevention and treatment of persistent infections. Developing algorithms for AMR

prediction could be of great clinical importance, offering a more reliable and efficient alternative to traditional phenotyping methods and potentially contributing to the discovery of novel AMR pathways.
The recent increase in available sequencing data and associated phenotypic information has laid the foundation for the development of predictive methods. However, predicting antimicrobial resistance in terms of minimum inhibitory concentration (MIC) is challenging, as it requires more nuanced analysis than simply categorizing strains as susceptible or resistant. We have developed a machine learning approach called ARSENAL (Antimicrobial ReSistance prEdictioN by mAchine Learning) to predict the MIC of several antibiotics based on genomic data. ARSENAL utilizes single-nucleotide polymorphisms (SNPs) and takes into account the genome structure (gene composition) and gene orthology links between strains of the same species.
We have shown in the application to the analysis of ~1300 strains of Streptococcus pneumoniae that our ARSENAL model demonstrates high predictive accuracy in determining the minimum inhibitory concentration of various antibiotics. Functional interpretation of the most predictive features confirmed the biological relevance of the ARSENAL model, highlighting its capacity to identify key genetic determinants of AMR.
ARSENAL is a novel machine learning approach for predicting antimicrobial resistance based on genomic data. By leveraging SNPs, genome structure, and gene orthology information, ARSENAL offers a powerful tool for understanding and predicting AMR, with the potential to guide clinical decision-making and contribute to the development of novel therapeutic strategies to combat the growing threat of multidrug-resistant infections."

30. Modulation of enniatin yield in *Fusarium avenaceum*: a focus on oxidative stress

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Mycologie et Sécurité Alimentaire - MycSA - UR 1264 – INRAE

Grain contamination by mycotoxins has a direct impact on the quality of cereals and their by-products, and can be the source of major health and economic issues. Among the mycotoxins of greatest concern are those produced by *Fusarium* species. Some of these toxins have been widely studied and are subject to European regulations. Other so-called "emerging" mycotoxins, such as enniatins, are poorly documented and knowledge on the factors and mechanisms modulating their biosynthesis is insufficient to allow defining efficient control strategies.
While many studies have shown oxidative stress (OS) to be a determining factor in the accumulation of regulated mycotoxins, the impact of this factor on enniatin yield is yet to be investigated.
Therefore, the present research project aims to study the impact of OS on the modulation of enniatins in *Fusarium avenaceum*, and to decipher the underlying molecular mechanisms. To do so, a panel of 12 *F. avenaceum* strains will be investigated for their tolerance to exogenous H2O2, as well as their growth and enniatin production under OS conditions. As AP-1 and Skn-7 are two transcription factors known to be associated with OS response, KO and constitutive overexpressing mutants for these genes will be generated using homologous recombination. The present study will lead to a better understanding of enniatin yield modulation by abiotic parameters such as

oxidative stress, as well as bring new insights on *F. avenaceum* diversity.

31. Adenovirus spreading and restriction factors in primary bronchial epithelia

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Introduction: Adenoviruses are considered respiratory viruses infection the upper respiratory tract causing mostly mild symptoms. The relatively mild disease progression in most cases is owed to life-long immune control following primo infections during childhood. However, some genotypes are associated with more severe respiratory infections coinciding with the release of proinflammatory cytokines. We would like to understand how adenovirus infections spread in bronchial epithelia and what intrinsic parameters and mechanisms control the spread. To address this question, we established a model of primary bronchial epithelia grown at the air liquid interphase derived from lung brushes of adult vs. child donors. We monitor the spread of viruses using live cell imaging and fluorescence microscopy at different stages of infection. Furthermore, we measure epithelia integrity together with apical and basolateral virus release over time to understand the infection dynamics. To understand the epithelial response to infection we further monitor cytokine release and the transcriptional response of the epithelia using scRNA-Seq.
Results: Adenovirus infection of epithelia progresses from established infection foci. Those foci develop into macroscopic lesions at defined time points after infection. Lesions appear in both age groups and coincide with decrease in transepithelial electrical resistance showing the disruption of the epithelial integrity. Viral spread occurs initially via the apical side of the epithelia but proceeds to the basolateral side once the epithelia is damaged. Some donors show a clear delay in lesions appearance, reminiscent of an increased intrinsic resistance to infection progression."

32. Étude en temps réel de la localisation de l'ADN viral du Prototype Foamy Virus

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Le Prototype Foamy Virus (PFV) est un rétrovirus résultant de la zoonose d'un Foamy Virus Simiens (SFV) chez l'Humain. Rétrovirus unique en son genre, il est classé dans la sous-famille différente des rétrovirus dit « conventionnels ». Les particularités des Foamy Virus en font des modèles attractifs pour le développement de nouvelles plateformes de thérapie génique et virothérapie oncolytique.
Le laboratoire a montré que lors de l'infection, le PFV se localise au centre organisateur des microtubules (MTOC) en l'attente d'une division cellulaire. Lors de la mitose, il s'encre ensuite à la chromatine via la protéine Gag, protéine cruciale dans la sélection des sites d'intégration de l'ADN viral. Notre problématique aujourd'hui est de pouvoir

déterminer la dynamique d'accès de l'ADN viral du MTOC aux chromosomes afin de mieux appréhender les mécanismes moléculaires régulant l'invasion de la chromatine de l'hôte par ce virus.
Suite au succès de la technologie ANCHORTM sur le VIH-1 qui permet un suivi de l'ADN viral en temps réel, nous avons entrepris la mise en place de ce système à notre modèle PFV. La technologie s'appuie sur la protéine OR3 fusionnée à la GFP qui présente la capacité de lier et s'oligomériser sur une séquence spécifique appelée ANCH. En ajoutant cette séquence dans le génome viral nous pouvons avoir ainsi un suivi des virus au sein des cellules vivantes afin de pouvoir étudier les transactions orchestrées lors du « trafficking » intracellulaire de ce virus."

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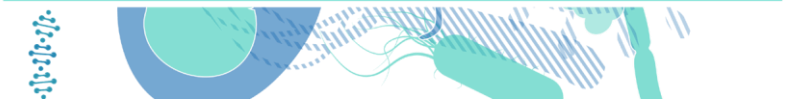

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