Dear colleagues,

We are delighted to invite you to the μNet ZOOM-seminars on **May 13th and 14th, 2024**. Save the date, come listen to the **19 speakers**. It will be a unique opportunity to listen to microbiologists from all over France and get your fill of great science.

Register here >> **https://micronet2024.sciencesconf.org/**

Abstracts of the seminars are listed below.

See you soon!

The μNet organizing committee.
https://sites.google.com/view/microbionetwork

Program at a glance

Monday, May 13

9:30 am – 9:40 am	**Welcome speech**
9:40 am – 12:30 pm	**Session 1** (Chairs : μNet Marseille)
9:40 am – 10:05 am	**Julien HERROU** (Marseille, LCB-IMM) "A Tad-like apparatus is required for contact-dependent prey killing in the predatory social bacteria *Myxococcus xanthus"*
10:05 am – 10:30 am	**Sylvain DURAND** (Paris, IBPC-UMR8261**)** "RNase J2 is involved in *Bacillus subtilis* lifestyle decision**"**
10:30 am – 10:55 am	**Erwan GUEGUEN** (Lyon, UMR5240 MAP, Univ Lyon, UCBL, CNRS, INSA Lyon) "A natural single nucleotide mutation in the small regulatory RNA ArcZ of *Dickeya solani* switches off the antimicrobial activities against yeast and bacteria"
10:55 am – 11:15 am	**Break**
11:15 am – 11:40 am	**Alexandre SMIRNOV** (Strasbourg, UMR7156 – GMGM) "Nearly universal ribosome isoaspartylation by two ancient enzymatic families"
11:40 am – 12:05 pm	**Zeynep BAHAROGLU** (Paris- Institut Pasteur - Unité Plasticité du Génome Bactérien) "Epitranscriptomic response to antibiotics in *Vibrio cholerae*"
12:05 pm – 12:30 pm	**Philippe REMIGI** (Toulouse, LIPME - CNRS-INRAE) "Experimental evolution of new plant symbionts: selective forces and genetic basis"
12:30 pm – 1:30 pm	**Lunch Break**
1:30 pm – 4:45 pm	**Session 2** (Chairs : μNet Toulouse)
1:30 pm – 1:55 pm	**Nicolas KINT** (Paris - Centre de Recherche des Cordeliers - UMR 1138) "Extracellular transfer of a conserved polymerization factor for multi-flagellin filament assembly in *Caulobacter"*
1:55 pm – 2:20 pm	**Juliana ALMARIO** (Lyon, Microbial Ecology Lab, CNRS, University of Lyon) "Adapting to symbiosis loss: uncovering new microbial associations in non-mycorrhizal plants"
2:20 pm – 2:45 pm	**Johannes STUTTMANN** (Saint-Paul-lez-Durance, LEMIRE - UMR7265 BIAM, CEA) "Beautiful circles – Plasmids for everyone"
2:45 pm – 3:10 pm	**Olivier CUNRATH** (Strasbourg, UMR7242-BSC, University of Strasbourg, CNRS) "Nutrient availability shapes gut colonisation resistance"
3:10 pm – 3:30 pm	**Break**
3:30 pm – 3:55 pm	**Olaya RENDUELES-GARCIA** (Toulouse, UMR5100, LMGM-CBI-CNRS) "The extracellular capsule, a sweet determinant of gene flux in *K pneumoniae*"
3:55 pm – 4:20 pm	**Emilia MAURIELLO** (Marseille, LCB CNRS-IMM). "Type IV-Pili and exopolysaccharide: molecular insights into twitching motility of *Myxococcus xanthus"*
4:20 pm – 4:45 pm	**Jérôme JOSSE** (Lyon, CIRI, INSERM U1111, CNRS UMR5308, ENS Lyon, UCBL, StaPath Team) "Intraosteoblastic replication dynamics of *Staphylococcus aureus* and tolerance to antibiotics"
4:45 pm – 4:55 pm	**Closing remarks**

Tuesday, May 14

9:30 am – 9:40 am	**Welcome speech**
9:40 am – 12:30 pm	**Session 3** (Chairs : μNet Lyon)
9:40 am – 10:05 am	**David LALAOUNA** (Strasbourg, UPR9002 – IBMC, CNRS) "A novel manganese efflux pump in *Staphylococcus aureus* displays a role in Mn detoxification and protein metalation"
10:05 am – 10:30 am	**Alessandro PAGLIUSO** (Jouy-en-Josas, EpiMic Team, INRAE Micalis Institute) "Breaking the wall : emergence of cell wall-deficient dormant forms under nutrient starvation"
10:30 am – 10:55 am	**Priscilla BRANCHU** (Toulouse, IRSD**)** "Hijacking the production of a virulence factor to efficiently vaccine animals"
10:55 am – 11:15 am	**Break**
11:15 am – 11:40 am	**Sarah BIGOT** (Lyon, MMSB, CNRS-University Lyon) "CAAX protease and bacteriocin-processing (CPBP)-type intramembrane proteases mediate competitive transfer between conjugative plasmids"
11:40 am – 12:05 pm	**Daniel CHEVRIER** (Saint-Paul-lez-Durance, MEM - UMR7265 BIAM - CEA) "Synchrotron-based X-ray imaging as a tool to investigate biomineralizing microorganisms in native-state"
12:05 pm – 12:30 pm	**Imane EL MEOUCHE** (Paris, IAME UMR1137) "Antibiotic tolerance in *Escherichia coli*: *in vitro* and *in vivo* approaches"
12:30 pm – 12:40 pm	**Closing remarks**

Abstracts

speakers

Julien HERROU (Marseille, LCB-IMM) "A Tad-like apparatus is required for contact-dependent prey killing in the predatory social bacteria *Myxococcus xanthus*"

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Myxococcus xanthus is an environmental predatory bacterium commonly found in the soil where it develops. This model organism finds its nutrients by collectively hunting and attacking other microorganisms. *Myxococcus* cells move by thousands to invade a prey colony and digest it. Therefore, predation appears to be a dynamic process involving motility and prey cell lysis. How *Myxococcus* recognizes and kills a prey remains poorly understood. In the Mignot team, we recently discovered that, during predation, direct predator-prey contact plays an important role in the initial invasion of the prey colony and prey killing by *M. xanthus*. We also discovered that a Tad-like (**T**ight **ad**herence) apparatus, named the Kil system, is essential for predation and is subject to remarkable contact-dependent regulations (Seef S. *et al.* 2021). Upon contact with a prey, the Kil system assembles, supposedly allowing local delivery of a toxin by the system itself or by an associated uncharacterized system. Its activation also signals a transient pause of the motility complex until prey cell lysis is complete. This suggests that a signal transduction cascade links prey sensing to the Kil system assembly, which in turn blocks motility and activates a lytic mechanism. We are currently investigating the function of the Kil system in prey recognition, intoxication and motility regulation.

Sylvain DURAND (Paris- Institut de Biologie Physico-Chimique - Unité Expression Génétique Microbienne UMR8261) **"RNase J2 is involved in *Bacillus subtilis* lifestyle decision"**

Ribonucleases (RNases) play a key role in bacteria by permitting rapid adaptation to environmental changes via post-transcriptional control. Two ribonucleases play key roles in mRNA degradation in the Gram-positive model bacterium *Bacillus subtilis*, the endoribonuclease Y and the 5'-3' exoribonuclease J1. *B. subtilis* also encodes an ortholog of RNase J1, called RNase J2, that is mostly found in a complex with J1 in *B. subtilis*. Previous work has shown that a lack of RNase J2 (∆*rnjB*) increases the expression of a few genes belonging to the *sigD* regulon [1], which governs cell motility. In other Firmicutes, RNase J2 has been shown to be a major regulator of cellular mechanisms such as competence, biofilm formation and virulence [2-3]. However, only few direct targets of RNase J2 have been identified in these organisms and the role of RNase J2 in RNA degradation is unclear as it has 1000-fold less 5'-3' exoribonuclease catalytic activity compared to RNase J1.

To better understand the role of RNase J2 in *B. subtilis*, we decided to do a phenotypic characterization of a ∆*rnjB* mutant strain, identify direct targets of this RNase and understand the determinants of its specificity. As observed in other Firmicutes, we have shown that a deletion of the *B. subtilis rnjB* gene affects biofilm structure and motility of the strain. We have also seen that RNase J2 expression is shutdown during sporulation. Interestingly, the level of cyclic-di-GMP, a signalling molecule involved in bacterial behaviours is also affected in the absence of RNase J2. In addition, we were able to confirm that a deletion of RNase J2, J1 or both enzymes stabilize two mRNAs belonging to the SigD regulon, namely *cheV* and *motA* mRNAs, encoding proteins linked to chemotaxis and motility in *B. subtilis*. In contrast, the catalytic mutant of RNase J2 does not affect the stability of these mRNAs. This suggests that the RNase J1/J2 complex is required for the degradation of *motA* and *cheV* mRNAs and that catalytic activity is carried by RNase J1.

Overall, our data strongly suggest that RNase J2 is directly link to the lifestyle decision in *B. subtilis.* The enzyme is probably enabling RNase J1 to target specific mRNAs link to cell fate decision. The determinants of RNase J2 specificity are under investigation.

Keywords: Ribonucleases, *Bacillus subtilis*, lifestyles

References

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Erwan GUEGUEN (Lyon, UMR5240 MAP, Univ Lyon, Université Claude Bernard Lyon 1, CNRS, INSA Lyon) ”A natural single nucleotide mutation in the small regulatory RNA ArcZ of *Dickeya solani* switches off the antimicrobial activities against yeast and bacteria”

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The necrotrophic plant pathogenic bacterium *Dickeya solani* emerged in the potato agrosystem in Europe. All isolated strains of *D. solani* contain several large polyketide synthase/non-ribosomal peptide synthetase gene clusters. Analogy with genes described in other bacteria suggests that the clusters *ooc* and *zms* are involved in the production of secondary metabolites of the oocydin and zeamine families, respectively. A third cluster, named *sol* produces a new antibiotic, solanimycin, capable of killing many yeasts, including the human pathogen Candida albicans. In our study, we analysed the antimicrobial functions of these three PKS/NRPS clusters against bacteria, yeasts or fungi in different wild-type *Dickeya solani* isolates. Phenotyping and comparative genomics revealed that the small regulatory RNA ArcZ plays a major role in the control of the clusters *ssm* and *zms*, but not *ooc*. A single-point mutation, conserved in some *Dickeya* wild-type strains, including the type strain IPO 2222, impairs the ArcZ function by affecting its processing into an active form. Our study showed that single-nucleotide polymorphisms of sRNA encoding genes can have huge impacts on bacterial phenotypes. It is thus critical to pay attention to the allele diversity of sRNA genes.

Alexandre SMIRNOV (Strasbourg, UMR7156 – GMGM, University of Strasbourg, CNRS) “Nearly universal ribosome isoaspartylation by two ancient enzymatic families”

Ribosome biogenesis is a highly complex and universally essential process. The assembly of ribosomal RNAs with proteins into translationally active particles follows overall the same logic across all forms of life. However, bacteria (as well as their derivatives, mitochondria and plastids) and archaea (and their descendants, eukaryotes) employ largely different sets of factors to build their ribosomes. Some of these factors are broadly conserved within their corresponding domain of life and responsible for some key ribosome biogenesis events. Here, we show that two unrelated ancestral enzyme families operate respectively on bacterial- and archaeal-type ribosomes to introduce the same unusual and functionally essential protein modification. Through different enzymatic mechanisms, these factors install an isoaspartate residue (isoAsp) in a strategic region of the universally conserved ribosomal protein uS11, enabling a critical structural change and its stable incorporation into the platform of the nascent small ribosomal subunit (SSU). Using bacterial, mitochondrial and eukaryotic models, we show that the inability to install the isoAsp in uS11 precludes further SSU maturation and dramatically impairs translation. The uS11 isoaspartylation is so-far the only known protein modification that appears to be shared by nearly all existing ribosomes. The two enzymatic mechanisms of its installation thus represent a remarkable case of convergent evolution aimed at resolving the same structural biogenesis challenge in bacterial- and archaeal-type ribosomes.

Zeynep BAHAROGLU (Paris- Institut Pasteur - Unité Plasticité du Génome Bactérien) "Epitranscriptomic response to antibiotics in Vibrio cholerae".

Antimicrobial resistance develops as a major problem in infectious diseases treatment. Starting with a high-density Tn insertion library in *Vibrio cholerae* and following its evolution by TN-seq in the presence of sub-inhibitory concentrations of antibiotics, we have linked 23 tRNA and rRNA modification enzymes with specific responses to various antibiotics in *V. cholerae*. We further studied the molecular mechanisms underlying this specificity in the absence of *tgt*/queuosine (Q) modification. We showed that (i) the absence of Q impacts tyrosine codon decoding and leads to translational reprogramming in response to stress. (ii) A protein's codon usage bias can influence its translation in a Q modification dependent way. (iii) Candidate transcripts subject to modification tunable translation can be identified in silico based on their codon content in bacteria. Our results highlight the existence of an epitranscriptomic and translational control of the bacterial response to antibiotic stress.

Philippe REMIGI (Toulouse, Laboratoire des Interactions Plante-Microbe-Environnement (LIPME), CNRS-INRAE) "Experimental evolution of new plant symbionts: selective forces and genetic basis."

Rhizobia are soil bacteria that can form nitrogen-fixing symbioses with legume plants. In bacteria, the ability to establish these symbioses evolved repeatedly through horizontal transfer of key symbiotic genes. Yet, the factors that determine the success of these horizontal gene transfers*, i.e.* whether or not the acquired genes will indeed allow the recipient bacterium to become a rhizobium, are still unclear. To investigate this question, we have initiated a long-term experiment that aims to evolve new rhizobia in the laboratory. Symbiotic genes from the rhizobium *Cupriavidus taiwanensis* were transferred to a plant pathogenic bacterium, *Ralstonia solanacearum.* The resulting strain was evolved through repeated cycles of interaction with the legume plant *Mimosa pudica*, the original host of *C. taiwanensis.* Within 35 cycles, evolved bacteria did not become mutualists, since they are still unable to fix nitrogen, but they rapidly improved their ability to perform the initial steps of symbiotic infection. I will describe our recent work that investigates the selective forces that drove these phenotypic changes, as well as their genetic bases.

Nicolas KINT (Paris - Centre de Recherche des Cordeliers - UMR 1138) "Extracellular transfer of a conserved polymerization factor for multi-flagellin filament assembly in *Caulobacter"*

The bacterial flagellum is a sophisticated and powerful nanomachine that propels individual cells in liquid and semi-solid environments. The displacement is enabled by the rotary motion of a long (typically) external and hollow protein filament that is joined to the flagellar engine via a flexible hook structure. Flagellar polymerization factors (PFs), such as the FliD capping protein, progressively extend the flagellar filament from the hook by insertion of newly secreted unfolded flagellin(s) that they receive at the growing tip. However, most of flagellated alpha-proteobacteria do not encode FliD orthologs in their genomes suggesting that unknown FliD-independent capping or polymerization mechanism exists. As the polarized α-proteobacterium *Caulobacter crescentus* encodes no discernible sequence homolog of the prototypical FliD capping protein, it is unclear how the six flagellin paralogs are polymerized into the flagellar filament in this organism. Here, we unveil FlaY as a defining member of an alternative class of specialized flagellin PFs. FlaY binds flagellin and is secreted by the flagellar secretion apparatus, yet it can also promote flagellin polymerization exogenously when donated from flagellin-deficient cells, serving as a transferable, extracellular public good.

Juliana ALMARIO (Lyon, Microbial Ecology Lab, CNRS, University of Lyon) “Adapting to symbiosis loss: uncovering new microbial associations in non-mycorrhizal plants”

The microbiota can mediate host adaptation. Plants rely on the ancestral Arbuscular Mycorrhizal (AM) symbiosis to supplement their phosphorus nutrition. However, recent findings indicate that the AM symbiosis is not essential. Indeed, there are at least three ‘non-mycorrhizal’ plant families for which the loss of the AM symbiosis was not compensated by any major nutritional innovation that we know of. Certain non-mycorrhizal Brassicaceae were discovered to associate with new types of root endophytic fungi capable of transferring phosphorus to the plant, suggesting the existence of yet-unknown nutritional associations between non-mycorrhizal plants and their microbiota. Our aim is to uncover and describe these nutritional associations by combining metagenomics and isotope tracing methods. Our overarching hypothesis is that non-mycorrhizal plants adapted to the loss of the AM symbiosis by establishing new nutritional microbial partnerships promoting their nutrition.

Johannes STUTTMANN (Saint-Paul-lez-Durance, LEMIRE - UMR7265 BIAM - CEA Cadarache) “Beautiful circles – Plasmids for everyone”

Fueled by next generation sequencing, the complex microbiomes inhabiting the human gut or the plant rhizosphere were extensively characterized during the past decade. In a shift towards more detailed and mechanistic analyses, many labs have now generated culture collections, and often work with synthetic communities. However, our capacities for genetic manipulation of the diverse microbiota are limited. Efficient transformation procedures are established mainly for long-standing model strains, and systematic approaches for evaluating e.g. vector components are scarce. Along these lines, for example our lab has a collection of > 5000 plant root-associated bacterial strains, but only a handful are used for genetics, and we so far relied on a few classical cloning vectors. This hinders the implementation of new technologies, as for example CRISPR base editing. Therefore, we have now started to build a hierarchical toolkit, based on GoldenGate cloning, for bacterial manipulation. The underlying molecular grammar allows us to build transformation vectors from a library of components, and to charge these with a cargo of interest, e.g. a base editing system, once verified for functionality in a respective target organism. I will present our systematic approach to construct assembly, from vector components to multigene constructs, with the scope to find interested colleagues for their evaluation in new target organisms towards a community resource.

Olivier CUNRATH (Strasbourg, UMR 7242 – BSC, University of Strasbourg, CNRS) "Nutrient availability shapes gut colonisation resistance"

The mammalian gut is home to diverse bacterial species collectively known as the gut microbiota. The gut microbiota plays a pivotal role in the host's health. One major health benefit is the protection against invading pathogens, known as colonisation resistance. While the ability of the gut microbiome to protect against invading pathogens is well known, we only recently shed light upon the fundamental ecological principals governing this effect. Although individual key strains were incapable of offering colonization resistance on their own, our findings demonstrate that community diversity is essential for enabling these key bacterial species to confer resistance. This resistance was mainly driven by a more complete blocking of nutrient availability of the bacterial community[1]. While we initially focussed on the nutrient carbon source, our newest findings, focussing on metal micro-nutrient such as iron show that iron availability also strongly influence the invasiveness of bacterial enteric pathogens. Bacteria can use various strategies for acquiring iron, of which the secretion and subsequent acquisition of siderophores is one of the most efficient in iron limited conditions. Siderophores are small organic molecules with a very strong affinity for iron. While it has been shown that pathogens produce and use their own siderophores during their infectious life-cycle, most enteric pathogens are also able to exploit exogenous siderophores, siderophores produced by other micro-organisms. Here we show that such exogenously added siderophores can strongly influence pathogens invasiveness and that this is a molecular tightly controlled strategy. Gaining these ecological and molecular insights allow us to have a more complete understanding of pathogens strategy to invade and colonise the gut and helping us to increase the chances of a more efficient development of targeted antimicrobial strategies.

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Olaya RENDUELES-GARCIA (Toulouse, UMR5100, Laboratoire de Microbiologie et Génétique Moléculaires (LMGM), CBI, CNRS) "The extracellular capsule, a sweet determinant of gene flux in *K pneumoniae*"

Bacterial evolution is affected by mobile genetic elements like phages and conjugative plasmids, offering new adaptive traits while incurring fitness costs. Their infection is affected by the bacterial capsule. Yet, its importance has been difficult to quantify because of the diversity of confounding mechanisms in bacterial genomes such as anti-viral systems and surface receptor modifications. Swapping capsule loci between *Klebsiella pneumoniae* strains allowed us to quantify their impact on plasmid and phage infection independently of genetic background. Capsule swaps systematically invert phage susceptibility, revealing serotypes as key determinants of phage infection. Capsule types also influence conjugation efficiency in both donor and recipient cells, a mechanism shaped by capsule volume and conjugative pilus structure. Comparative genomics confirmed that more permissive serotypes in the lab correspond to the strains acquiring more conjugative plasmids in nature. The least capsule-sensitive pili (F-like) are the most frequent in the species' plasmids, and are the only ones associated with both antibiotic resistance and virulence factors, driving the convergence between virulence and antibiotics resistance in the population. These results show how traits of cellular envelopes define slow and fast lanes of infection by mobile genetic elements, with implications for population dynamics and horizontal gene transfer.

Emilia MAURIELLO (Marseille, LCB CNRS-IMM). "Type IV-Pili and exopolysaccharide: molecular insights into twitching motility of *Myxococcus xanthus".*

Authors: Shuanghong Xue, Camille Maille, Guiseppi A, Mignot T and **Mauriello Emilia**

Type Four Pili (T4P) are extracellular appendages that mediate several bacterial functions such as motility, biofilm formation, and infection. The ability to adhere to substrates is essential for all these functions. In *Myxococcus xanthus*, during twitching motility, the binding of polar T4P to components of the extracellular matrix induces pilus retraction and forward cell movement. Recently, a genetic screen allowed us to identify PilY1.1 and PilY1.3 as pilus proteins responsible for binding. A genetic and bioinformatic dissection of the PilY1 domains shows that PilY1 proteins are bifunctional, with a role in priming T4P extension mediated by their conserved C-terminal domain, and roles in adhesion mediated by N-terminal variable domains. PilY1.1 has a sugar-binding (lectin) domain, and PilY1.3 has a protein-binding (von Willebrand Factor A) domain at the N terminus. Finally, transcriptomic analyses show that HsfBA differentially regulates the expression of PilY1 proteins, suggesting that this transcriptional regulation of PilY1 homologs in response to unknown signals might allow the accessorizing of T4P tips with different modules, enabling twitching motility in the presence of alternative substrates and environmental conditions.

Jérôme JOSSE (Lyon, Centre International de Recherche en Infectiologie (CIRI), INSERM U1111, CNRS UMR5308, ENS Lyon, Université Claude Bernard Lyon 1, Staphylococcal Pathogenesis Team) "Intraosteoblastic replication dynamics of *Staphylococcus aureus* and tolerance to antibiotics"

Chronicization of *Staphylococcus aureus* bone and joint infections is associated with different physiopathological mechanisms such as biofilm formation, small colony variant phenotype and intracellular survival within osteoblasts, the bone forming cells. Once inside the osteoblast, *S. aureus* can stay quiescent without replicating or replicate, leading to host cell lysis. The intracellular non-growing *S. aureus* are particularly worrying as most antibiotics are inactive on non-replicating bacteria, allowing *S. aureus* to persist inside osteoblasts and act as a reservoir for a future relapse. In this context, we aim to determine the intraosteoblastic replicative dynamics of *S. aureus* and its impact in the presence or absence of antibiotics at clinically-relevant concentrations. To this purpose, we developed an automated live confocal microscopy approach that allows us to follow the replicative status of *S. aureus* inside osteoblasts. In absence of treatment, we observed that 45% of osteoblasts infected with *S. aureus* SH1000 contain exclusively non-replicative *S. aureus* 24h post-infection, whereas 45% and 10% of infected osteoblasts contain respectively fast and slow replicative bacteria. These percentages vary depending on the strain used for the infection, highlighting that the intracellular replication dynamics is strain-dependent. Treatment with rifampicin led to 100% of infected osteoblasts containing exclusively non-replicative *S. aureus*, for all the tested strains. These non-growing intracellular *S. aureus* regrew on agar plates after cell lysis, with a globally reduced colony size for rifampicin-treated *S. aureus* compared to non-treated, suggesting that these non-growing *S. aureus* are intracellular persisters.

David LALAOUNA (Strasbourg, UPR9002 – IBMC, University of Strasbourg, CNRS) "A novel manganese efflux pump in *Staphylococcus aureus* displays a role in Mn detoxification and protein metalation"

Manganese (Mn) is an essential micronutrient involved in critical biological functions in *Staphylococcus aureus*. Paradoxically, Mn can also be toxic when in excess. As intracellular Mn homeostasis is required for survival and optimal virulence during infection, *S. aureus* employs diverse import and export systems to deal with fluctuations in Mn availability. In response to Mn scarcity, the Mn importers MntABC and MntH are synthetized, being under the control of the Mn-sensing transcription factor MntR. In contrast, the Mn exporter MntE is activated when Mn levels are toxic. Here, we identified an additional Mn exporter called MntY (for Mn transporter Y). In addition to its role in protecting cells from Mn toxicity, MntY participates in the metalation of Mn-dependent exoenzymes. Unlike the established Mn efflux pump MntE, MntY is still expressed at low Mn levels, strongly suggesting distinct roles. We notably observed that MntY is required for the function of the lipoteichoic acid synthase (LtaS), a membrane-localized enzyme with an extracytoplasmic Mn-dependent active site. Remarkably, we revealed that MntY is a conditionally essential Mn efflux pump in *S. aureus*. As maintaining the proper balance of Mn is crucial for bacterial survival, MntY could be a promising new antimicrobial target against *S. aureus*.

Alessandro PAGLIUSO (Jouy-en-Josas - Epigenetics and Cellular Microbiology (EpiMic) Team - INRAE Micalis Institute) "Breaking the wall: emergence of cell wall-deficient dormant forms under nutrient starvation"

Bacteria can adapt to nutrient starvation by hibernating in a viable but non-culturable (VBNC) state. VBNC bacteria are alive and metabolically active, but have lost the capability to grow in routine culture media. Although hundreds of bacterial species have been reported to transition into the VBNC state, the underlying molecular mechanisms remain largely elusive. We have recently characterized the VBNC state in the Gram-positive pathogen *Listeria monocytogenes*. By combining fluorescence microscopy, cryo-electron tomography with powerful genetic and biochemical approaches, we discovered that starvation in mineral water drives *L. monocytogenes* into a VBNC state via a unique mechanism of cell wall shedding that generates osmotically stable cell wall-deficient coccoid forms. Our results shake the longstanding assumption of the cell envelope as a fundamental bacterial component.

Priscilla BRANCHU (Toulouse, Institut de Recherche en Santé Digestive (IRSD)) “Hijacking the production of a virulence factor to efficiently vaccine animals”

Outer membrane vesicles (OMVs) produced by Gram negative bacteria play a crucial role in several bacterial functions, such as waste export or nutrient acquisition, as well as virulence for some pathogenic bacteria *via* toxins or effectors transport to target the host cells. In the lab, we discovered an enzyme able to boost the production of OMVs and modify OMVs pro-inflammatory properties. This enzyme is encoded by the gene *hlyF,* located on a large virulence plasmid and has been used as an epidemiological marker of avian pathogenic *Escherichia coli* (APEC) for decades although its role and mode of action have never been investigated in details. The gene encoding *hlyF* is also found in some other pathovars of *E. coli* such as neo-natal meningitis associated *E. coli*, uropathogenic *E. coli* (UPEC)*,* the emerging O80:H2 serotype of enterohaemorrhagic *E. coli* and also adherent-invasive *E. coli*. We demonstrated that OMVs produced by an *E. coli* strain expressing *hlyF* specifically impede the autophagy flux and activate the non-canonical inflammasome pathway of eukaryotic cells. We also showed that these OMVs have the ability to disrupt the epithelial barrier. In parallel, we demonstrated that *hlyF* is a virulence factor as its expression in an APEC strain induces a higher rate of mortality in a chicken model of infection and similarly, its expression in an UPEC strain is associated with urosepsis in a mice model of urinary tract infection. However, we were able to take advantage of this virulence factor to overproduce OMVs with pro-inflammatory properties to produce a vaccine with OMVs directly from an APEC strain carrying *hlyF*. We vaccinated chickens with OMVs from this strain and completely protected chickens against this strain after a challenge. In conclusion, HlyF is an example of the yin-yang effect of certain pathogenic factors whose deleterious effects can be redirected to treat or protect humans or animals.

Sarah BIGOT (Lyon, Laboratory of Molecular Microbiology and Structural Biochemistry (MMSB), CNRS-University Lyon) "CAAX protease and bacteriocin-processing (CPBP)-type intramembrane proteases mediate competitive transfer between conjugative plasmids"

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The primary mechanism of genetic material exchange is bacterial conjugation, where DNA, mainly conjugative plasmid, is transferred through direct contact between a donor cell and a recipient cell. Conjugative plasmids are in conflict with co-resident competitors and have developed a variety of mechanisms to successfully promote their dissemination. Among these, fertility inhibition (FI) systems prevent the transfer of unrelated co-resident conjugative plasmids. Although many FI systems have been identified, their mechanism and functional roles are diverse and poorly understood *(1)*. This is notably the case with the Tir protein, identified in 1985 from the plasmid R100 of the IncFII incompatibility group, as a FI factor responsible for inhibiting the transfer of the plasmid RP4 an IncP plasmid *(2)*.
This study elucidates the molecular mechanism underlying the inhibitory effect of Tir. Tir, belonging to the CAAX protease and bacteriocin-processing (CPBP)-type intramembrane proteases, was confirmed as an inner transmembrane protein. Through experimentations with a range of Inc plasmids, we demonstrate that Tir selectively targets IncP and IncW plasmids, thereby impacting their transfer efficiency. Point mutations of conserved residues critical for CPBP proteases enzymatic function resulted in the loss of inhibition of IncP and IncW plasmid transfer, indicating the essentiality of Tir's catalytic activity for affecting the transfer efficiency of targeted co- resident plasmids. Further exploration into the mechanisms through which Tir inhibits plasmid transfer revealed its interaction with the VirB10 protein, a component of the type IV secretion (T4SS) machinery. Evidence suggests that Tir's proteolytic activity impedes the interaction between VirB10 and its T4SS partners, thereby hindering productive DNA transfer within the secretion channel. The conservation of Tir homologue across various conjugative plasmids from different incompatibility groups suggests a broad use of Tir as a mechanism to outcompete co-resident plasmids.

1. M. Getino, F. de la Cruz, Natural and Artificial Strategies To Control the Conjugative Transmission of Plasmids. *Microbiol Spectr* **6** (2018).
2. K. Tanimoto, T. Iino, H. Ohtsubo, E. Ohtsubo, Identification of a gene, *tir* of R100, functionally homologous to the F3 gene of F in the inhibition of RP4 transfer. *Mol Gen Genet* **198**, 356–357 (1985).

Daniel M. CHEVRIER (Saint-Paul-lez-Durance, MEM - UMR7265 BIAM - CEA Cadarache) "Synchrotron-based X-ray imaging as a tool to investigate biomineralizing microorganisms in native-state"

Interfacing microfluidic environments with nano-scanning X-ray microscopy techniques presents new possibilities to retrieve the intracellular composition of microorganisms in the native state. This work demonstrates this experimental potential at synchrotron facilities by measuring the intracellular iron composition via X-ray fluorescence (XRF) of hydrated magnetotactic bacteria containing biominerals of nanocrystalline magnetite. Both commercial and custom-made microfluidic environments are employed for nano-scanning XRF measurements while the latter is used to follow magnetite biomineralization in situ. The effect of X-ray beam damage to bacterial cells was also investigated to take into consideration the limitation of in situ studies. Future developments in liquid cell construction and radiation dose-limited measurement strategies will enable native-state imaging for a wide-range of microorganisms and facilitate "on-chip" experiments to be conducted at the synchrotron.

Imane EL MEOUCHE (Paris, Infection Antimicrobials Modelling Evolution, IAME UMR1137) "Antibiotic tolerance in *Escherichia coli*: *in vitro* and *in vivo* approaches"

Antibiotic resistance is a major health problem. However, even without resistance, many infections are hard to treat and tend to relapse. Even without increase in the minimal inhibitory concentrations, cells can survive longer even in presence of lethal concentrations of bactericidal antibiotics (tolerance and persistence). In this talk, I will discuss the different *in vitro* and *in vivo* approaches that we are currently using to understand why bacteria that are sensitive to antibiotic survive. We focus on *Escherichia coli*, a commensal, opportunistic pathogen and a major cause of urinary tract infections. I will describe our experiments that aim to decipher mechanisms of antibiotic tolerance in commensal and pathogenic extraintestinal *E. coli* strains using physiologically relevant conditions such as urine. We focus on urinary tract infections, very common and an example where recurrences and increased antimicrobial resistance occur. In the lab we use a murine pyelonephritis model allowing us to understand the interplay between the cell's physiology and growth in the different niches of infection. I will discuss our advances in using engineered circuits to study the heterogeneity of *E. coli* growth and antibiotic response *in vivo*. Finally, I will present our collection of strains causing recurrent infections that we are studying to identify whether microbiological factors are involved in treatment failure.