Phages in GRENOBLE

Fifth symposium of the French PHAGE Network

8-9 Octobre 2019

EPN Campus,
Institut de Biologie Structurale,
Grenoble

Sessions

- Ecology & Evolution
- Structure & Assembly
- Phage-host interaction
- Therapy & Biotechnology

Invited speakers

- Evelien Adriaenssens,
  Quadram Institute, Norwich, UK
- Stefanie Barbirz,
  Universität Potsdam, DE
- Claire Geslin,
  Microbiologie des Env. Extrêmes, FR
- Călin Guet,
  Institute of Science & Technology, AT

Scientific committee

Cécile Breyton
Marianne De Paepe
Rémy Froissart
Nicolas Ginet

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Tuesday, October 8, 2019

8h00	**Registration**	
8h30	**Cécile Breyton**	**Welcome**
Ecology & Evolution		***Chair: Damien Piel***
8h45	**Evelien Adriaenssens**	***Phage Taxonomy: What, how and why?***
9h20	Frédérique Le Roux	*Spatio-temporal dynamics of Vibriophages, a first step toward controlling pathogenic Vibrio populations with eco-friendly weapons*
9h40	Eugen Pfeifer	*Phage-plasmids: bridges between phages and plasmids?*
10h00	Cécile Philippe	*Phage proteins promote plasmid stability*
10h20	Coffee-break	
Ecology & Evolution		***Chair: Jack Dorling***
10h50	Marie-Agnès Petit	*Pig fecal virome contigs are poor in antibiotic resistance genes and rich in Inoviridae*
11h10	Clara Torres-Barceló	*"Jack of all strains, master of none"? Host range and efficacy in bacteriophages of a phytopathogenic bacterium*
11h30	Hélène Chabas	*What drives the survival of phages in host populations carrying an adaptive immune system?*
11h50	Flash posters	Even numbers
12h10	Lunch	
13h10	**Poster session**	Even numbers
Phage Therapy & Biotechnology		***Chair: Fernando Clavijo***
14h30	**Claire Geslin**	***Viruses of (hyper)thermophilic archaea and bacteria isolated from deep-sea hydrothermal vents***
15h05	David R. Olivenza	*Design and optimisation of phase variation-based biosensors capable of highly sensitive bacteriophage detection as well as phage receptor discrimination.*
15h25	Ildikó K. Nagy	*Comparative genomics of Xanthomonas oryzae pv. oryzae bacteriophages*
15h45	Alexandre Bleibtreu	*Traitement de sauvetage d'un empyème extradural par phagothérapie personnalisée en association avec antibiothérapie intraveineuse*
16h05	Raphaëlle Delattre	*« Phagothérapie - Retour d'expérience et perspectives » - Compte-rendu du CSST de l'ANSM*
16h25	Coffee Break	
Structure & Assembly		***Chair: Müge Senarisoy***
16h55	**Stefanie Barbirz**	***Bacteriophage genome transfer mechanisms at the Gram-negative cell wall***
17h30	Romain Linares	*Structural study of the tail tip of siphophage T5*
17h40	Séraphine Degroux	*Functional and structural study of theT5 bacteriophage immunity protein Llp*
18h00	Flash posters	Odd numbers
18h20	**Poster session**	Odd numbers
19h45	Wine & Cheese	

Wednesday, October 9, 2019

Phage-Host Interaction		***Chair: Maud Billaud***
8h30	**Calin Guet**	***The cost of immigration control and the benefits of illegal immigration***
9h05	Marie Vasse	*Phage-associated modifications of bacterial lifecycle*
9h25	Amel Chaïb	*Lysogeny in the Lactic Acid Bacterium Oenococcus oeni Is Responsible for Modified Colony Morphology on Red Grape Juice Agar*
9h45	Pauline Misson	*Characterization of E. coli LF82 prophages and study of their impact on the macrophage-induced persistence of their host*
10h05	Coffee-break	
Phage-Host Interaction		***Chair: Adelaïde Renard***
10h35	Mehdi El Sadek Fadel	*Molecular mechanisms of selective viral DNA recognition and packaging of the bacteriophage SPP1*
10h55	Luis Ramirez	*Bacteriophage T5 early genes: a model to dissect first host-phage interactions.*
11h15	Naoual Derdouri	*AppY, a new RpoS regulator from prophage origin*
11h35	Audrey Labarde	*Mechanisms of replication for an efficient multiplication of bacteriophage SPP1 DNA during viral infection*
11h55	Quentin Lamy-Besnier	*Viral Host Range database (VHRdb), an online resource to collect, browse and analyse the host range of viruses*
12h15	Kovacs Tamas	*Presentation of the EuroXanth COST action – planning a COST action on bacteriophage research and application*
12h30	Lunch	
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Oral Presentations

Phage Taxonomy: What, how and why?

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2 : University of Guelph

It is almost a cliché to state that bacteriophages are the most abundant biological entities on the planet. The tailed phages belonging to the order *Caudovirales* are perhaps the most diverse with a genome size range spanning more than an order of magnitude. Historically, these phages were classified into families based on morphological analysis of their tail structure, resulting in the families *Myoviridae*, *Siphoviridae* and *Podoviridae*. This classification scheme has come under pressure with thousands of bacteriophage sequences deposited in public databases in recent years, showing that there is little to no sequence homology between members of the same family.
The ICTV (International Committee on Taxonomy of Viruses) Bacterial and Archaeal Viruses Subcommittee is formally tasked with the classification of bacteriophages and bringing order to the chaos of unclassified genomes. The subcommittee has started dismantling the morphology-based families in favor of genome-based ones where we use an ensemble of genomic, proteomic and phylogenetic methods to delineate families and their internal structure. Using the case study of a group of large myoviruses, the spounaviruses, now grouped into a new family called *Herelleviridae*, I will present how we are envisioning a large-scale, genome-based taxonomic classification that can robustly be carried into the future, fit for high-throughput and metagenomic analyses.

Spatio-temporal dynamic of Vibriophages, a first step toward controlling pathogenic Vibrio populations with eco-friendly weapons

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Recent analyses of microbial community dynamics in the coastal ocean have revealed rapid turnover and short-lived blooms of different bacteria and eukaryotes (Martin-Platero *et al.,* 2018). Viruses, as an important group of predators of bacteria in the ocean, are thought to play an important role in controlling fine-scale blooms within these communities – yet, how these dynamics are structured in the environment remains an open question. Here, we focus on *Vibrio crassostreae,* a species of known oyster pathogens (Lemire *et al.,* 2015; Bruto *et al.,* 2017), to address the questions of: i) whether phages are major drivers of microbial abundance and diversity, and ii) how phages evolve in response to vibrio resistance. Using a time series sampling approach to track the dynamics of *Vibrio* and their phages at two distant sites (Brest and Sylt) we show that Vibriophage blooms correlate with decreases in *Vibrio* abundance in the environment over time. Investigating the host ranges of Vibriophages isolated from these samples we find, in an all-by-all host range assay, that sympatric killing of bacteria by phages (Brest/Brest; Sylt/Sylt) is more frequent than allopatric killing (Brest/Sylt), strongly suggesting local adaptation. Preliminary genome analyses of these *Vibrio* and phage isolates provides insight into the mechanistic foundations of virus-host interactions and patterns of adaptation over time in natural environments. Finally, the characterization of three broad host phages discovered in this work provides the basis for the rational design of a phage combination for use in phage therapy against an important group of oyster pathogens.

Phage-plasmids: bridges between phages and plasmids?

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Phages and plasmids are two major groups of mobile genetic elements (MGEs) shaping bacterial evolution. Although literature distinguishes them clearly, several MGEs were reported to be plasmids encoding *bona fide* phage genes (or vice versa). The two enterobacteriophages P1 and N15 are the most prominent examples of these so-called "phage-plasmids". However, in the recent years more and more phages and plasmids were isolated [1] with similar patterns and, so far, the distribution, the physiology and the impact of phage-plasmids are poorly understood. In our work, we study known phage-plasmids, focus on the identification of novel ones and examine how they impact bacterial evolution.

To identify phage-plasmids, we screened phage and plasmid databases for typical phage characteristics in plasmids and vice versa. Plasmid functions were searched by an in-house annotation pipeline [2] based on hidden Markov models of protein profiles. For phage functions, we selected a specific set of pVOG profiles representing the most comprehensive database (derived from ~3000 phage genomes) [3]. In combination with a supervised ensemble-learner, we predicted ~500 phage-plasmids mainly in γ-proteobacteria and Firmicutes. The clustering of these mobile elements based on the genome repertoires resulted in a homology network allowing the identification of phage-plasmid families. Strikingly, a few families encode many virulence and resistance traits. We are now studying the genomic organization and context of phage-plasmids to understand their evolution. Our work provides the first global insight on elements that belong to a hitherto underestimated type of MGE at the interface between phages and plasmids.

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[2] S. S. Abby, B. Neron, H. Menager, M. Touchon, and E. P. C. Rocha. PLoS ONE. 2015 Oct; E 9(10): e11072
[3] A. L. Grazziotin, E. V. Koonin, and D. M. Kristensen, Nucleid Acids Res. 2017 Jan; 45(Database issue): D491–D498

Phage proteins promote plasmid stability

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The dairy bacterium *Streptococcus thermophilus*is among the most important lactic acid bacteria (LAB) used to manufacture yogurt and cheese. It has a long history of safe use and thus possesses the GRAS (generally recognized as safe) status. Besides its utility in industrial food fermentation, LAB are generating interests as potential host for food-grade protein or metabolite production through genome engineering [1,2]. LAB are attractive because of their biotechnological performances and their food origin. Food-grade expression relies on GRAS organisms and on non-dependence on antibiotic markers. To do so, replicating plasmids are often used to introduce genetic material into LAB cells [3–5]producing many copies of the gene of interest and enhancing the desired phenotype. Maintenance of food-gradevectors into the cells are generally achieved by auxotrophic complementation, sugar utilization [6], and more recently, the use of CRISPR-Cas technology [7,8].In our work, we uncover streptococcal phage proteins that increase plasmid stability after repeated culturing without selective pressure. We demonstrate that this plasmid stability phenotype is not strain-dependent using different industrial strains.In addition, we address the relevance of this strategy for a potential application in dairy fermentation, in a context of phage-risk control, providing a new tool for food-grade vector applications.

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2. Tagliavia, M.; Nicosia, A. Microorganisms2019, 7, 116.
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Pig fecal virome contigs are poor in antibiotic resistance genes and rich in Inoviridae

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Our previous search for antibiotic resistance genes in either phage genomes or virome contigs led to the conclusion that they were infrequent [1]. However, since then, some reports suggested that pig fecal samples were rich in viral ARG genes, although conclusions were uncertain, as the samples were contaminated by bacterial DNA [2,3].
To maximize the chances to detect such ARG in phage contigs assembled from viromes, we selected 14 pig fecal samples, 5 from piglets and 9 from adults, raised in 14 French farms using more or less antibiotics, 3 of them with a bio label. Starting from 2 gr fecal samples, fecal resuspensions were centrifuged to remove bacteria, supernatants were then PEG concentrated, viral particles and vesicles were separated on iodixanol gradients, and the viral band was recovered and treated with S1 nuclease to remove extraneous DNA fragments. Finally, viral DNA was extracted by classical phenol-chloroform treatment followed by ethanol precipitation. Some samples had too little DNA for Trueseq libraries (124-1400 ng), so an amplification step with the Genomiphi kit was performed. Pair-end sequencing was performed on an Illumina Hiseq platform (2 x 150 nt), and high depth of sequencing was chosen (50 million reads per sample). Bacterial contamination, assessed by the frequency of reads mapping onto the 16S Silva database, was low ($2.8x10^{-5}$ to $4x10^{-8}$ depending on each sample), and always 10-fold lower than the recommended threshold o f 2 10-4. Reads were trimmed, dereplicated and assembled with Spades. An average of 577 (+/- 396) contigs > 2kb (size cutoff = smallest viral genome were obtained in each virome. For these contigs, gene calling was performed with Prokka, and a total of 101,249 ORF were obtained. No ARG was detected using Resfam core database and the gut_ga option [as in 1] in the 9 adult samples (gene frequency below $1.6x10^{-5}$, same order of magnitude as in [1]). However, in the 4 piglet samples collected in non bio farms, 8 ARG genes (on 7 contigs) were found (gene frequency $4x10^{-4}$). One of the contigs corresponded to a *Clostridium difficile* plasmid. Interestingly, in the 6 other contigs (size > 13 kb) no phage structural gene was found. These contig may correspond to transduced bacterial DNA. We conclude that the phage contigs reconstructed from pig virome samples, as all other so far characterized, are poor in ARG.
Inoviridae are much more prevalent in viromes than previously thought [4]. This is particularly true for pig fecal samples. Using the inovirus_detector program of Roux et al. [4], we detected 286 complete Inoviridae genomes (3-11 kb long). Their main properties will be presented.

[1] Enault, F., Briet, A., Bouteille, L., Roux, S., Sullivan, M.B. and Petit, M.A. (2016) Phages rarely encode antibiotic resistance genes: a cautionary tale for virome analyses. *ISME J*.
[2] Calero-Caceres, W., Ye, M. and Balcazar, J.L. (2019) Bacteriophages as Environmental Reservoirs of Antibiotic Resistance. *Trends in microbiology*, **27**, 570-577.
[3] Debroas, D. and Siguret, C. (2019) Viruses as key reservoirs of antibiotic resistance genes in the environment. *ISME J*.
[4] Roux, S., Krupovic, M., Daly, R.A., Borges, A.L., Nayfach, S., Schulz, F., Sharrar, A., Matheus Carnevali, P.B., Cheng, J.F., Ivanova, N.N. *et al.* (2019) Cryptic inoviruses revealed as pervasive in bacteria and archaea across Earth's biomes. *Nature microbiology*.

“Jack of all strains, master of none”? Host range and efficacy in bacteriophages of a phytopathogenic bacterium

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Natural bacteriophages (phages) hold the promise of being the perfect hunters of pathogenic bacteria in a wide range of applications, from medicine to industry or agriculture. As a consequence of the extreme specificity of most phages, a broad host range is the main criterion of phage selection in therapeutic cocktails, which are used with varied results. Including other approaches could refine the selection of candidate phages, such as precise measurement of phage efficacy or bacterial resistance frequency to each phage and its genetic base. Besides, evolutionary trade-offs among the different phage characteristics should be evaluated to improve the durability of many phage applications.

Bacterial wilt caused by the *Ralstonia solanacearum* species complex (*Ralstonia* spp.) is among the most important plant diseases worldwide, severely affecting a high number of crops and ornamental plants in tropical regions. The aim of the present study was to isolate and characterize new phages capable of infecting the *Ralstonia* spp., to be used as biocontrol tools. Along the way, evolutionary questions were addressed on the host range and efficacy of the isolated phages. The epidemiology of this bacterium has been extensively studied in the South West Indian Ocean (SWIO), establishing that, in Reunion island, three phylotypes are present, but a single haplotype from phylotype I is very abundant (60%). In Mauritius, only phylotype I has been reported but plenty of different haplotypes of *Ralstonia* spp. infect plants in the island, displaying a more complex epidemiology.

Forty-two phages infecting *Ralstonia* spp. were isolated from agricultural samples collected in Mauritius and Reunion islands. Host range of phages was tested measuring their capacity of replication in 64 *Ralstonia* spp. strains, from local or international origin. We demonstrate that all phages preferentially attack the most abundant *Ralstonia* spp. phylotype in both islands (phylotype I), but harbor a high host range variability. Surprisingly, 18 phages are able to multiply in bacterial phylotypes never detected in the islands. This implies either that phylogenetically distant bacteria have common genetic features related to phage recognition, or that the epidemiological efforts made in the SWIO should continue. The studied phages also show differences in their efficiency at decreasing bacterial growth of the most problematic strains of phylotype I in both islands. Specifically, Reunion phages are relatively generalist but highly efficient at reducing bacterial density of the genetically homogeneous local *Ralstonia* spp. strains. Mauritius phages are highly specific and only efficient in some of the highly diverse *Ralstonia* spp. strains present in this island. Our results point out that host range should not be the unique criteria to select the phages to be used in cocktails. The level of efficacy at decreasing bacterial populations of each strain suggests that quantitative and varied measurements are necessary to assess “a good phage” candidate.

We are currently exploring the hypothesis that an evolutionary trade-off exists in *Ralstonia* spp. phages between efficacy and host range. This negative correlation has been proven in parasitic wasps and aphids, for example, but not in phages and bacteria to our knowledge. We are also performing molecular analysis of the isolated phages and we plan to evaluate the *in vivo* capacity of combinations of phages to protect tomato plants from the bacterial disease. Our study does not only involve fundamental ecological research, but also concrete practical perspectives.

What drives the survival of phages in host populations carrying an adaptive immune system?

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Phages are viruses that spread in prokaryotic populations. For a phage epidemic to occur, phages need to bypass host immunity. Especially, CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR Associated System) is a widespread prokaryotic adaptive immune system. This system acquires resistance by storing a short phage DNA sequence in the prokaryotic genome and using it as a guide to degrade phage DNA [1]. As this immunity relies on Watson-Crick pairing to confer immunity, a single mutation in the targeted phage sequence is sufficient to escape it [2]. Therefore, it was thought that coevolution between phages and CRISPR was a likely scenario and this coevolution was indeed described [3, 4, 5]. However, a recent study shows that in some cases, CRISPR leads the phage to extinction because it generates a large diversity of CRISPR-resistances that the phage cannot escape [6]. Why do some phage infections lead to large epidemics whereas others lead to phage extinction? We hypothesize that two parameters are key to predict the outcome of CRISPR-phage interactions: the probability that a prokaryote acquires a new CRISPR-resistance and the probability of phage escape evolution.

To explore this, we developed a stochastic model that describes CRISPR-phage interactions. We explore how various probabilities of phage evolution and of acquisition of new CRISPR-resistances modify the probability of phage extinction. We first show that a higher probability of CRISPR-resistance acquisition increases the probability of phage extinction, because it increases the level of CRISPR-resistance diversity. We also show that higher levels of phage evolution increase the probability of phage survival and the size of the epidemics, even when phage evolution is costly. As the probabilities of phage evolution and CRISPR-resistance acquisition are under selection, these results may imply that CRISPR-resistance acquisition is a driver of the evolution of phage mutation rates.

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[5] Common J, Morley D, Westra ER and van Houte S. *Philos Trans R Soc Lond B Biol Sci*. 13;374(1772):20180098 (2019).
[6] van Houte S, Ekroth AK, Broniewski JM, Chabas H, Ashby B, Bondy-Denomy J, Gandon S, Boots M, Paterson S, Buckling A and Westra ER. *Nature*. 21;532(7599):385-8 (2016).

Viruses of (hyper)thermophilic archaea and bacteria isolated from deep-sea hydrothermal vents

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Viruses have a potential to be powerful agents that drive evolution and adaptation of their cellular hosts even in extreme marine environments. Deep-sea hydrothermal vents represent one of the most extreme environments on Earth. These ecosystems are characterized by steep physicochemical gradients, high hydrostatic pressure, high to low temperatures, obscurity and the prevalence of chemosynthesis. Our knowledge of the viral diversity associated to microorganisms inhabiting these deep sea hydrothermal vents is still limited: only 10 viruses, 2 archaeoviruses and 8 bacterioviruses, were isolated and described; we have characterized five of them in the laboratory.

Two lemon-shaped viruses, PAV1 and TPV1, associated to hyperthermophilic anaerobic *Archaea*, *Thermococcales* order, have been well studied. The only two genes that are homologous between TPV1 and PAV1 encode proteins containing a concanavalin A-like lectin/glucanase domain that might be involved in virus–host recognition.

Methanogens represent another important group of archaea colonizing deep sea hydrothermal vents. Few viruses infecting methanogens have been discovered and none of them from an abyssal ecosystem. We characterized MFV1 (*Methanocaldococcus fervens* virus 1), the first head-tailed virus providing from a deep sea hyperthermophilic archaea.

In order to deepen our knowledge on the viral diversity of marine hydrothermal microorganisms, we have extended our investigation to the Bacteria domain. Indeed, by studying these two domains of life we will be able to better apprehend the viral diversity of these extreme ecosystems.

In particular, we characterized MPV1 that infects *Marinitoga piezophila*, a thermophilic, anaerobic and piezophilic bacterium. MPV1 is a temperate siphovirus with a 43.7 kb genome. Surprisingly, we found that MPV1 virions carry not only the viral DNA but preferentially package a plasmid of 13.3 kb (pMP1) also carried by *M. piezophila*. This ‘ménage à trois' highlights potential relevance of selfish genetic elements in facilitating lateral gene transfer in the deep-sea biosphere.

Comparative studies of these mobile genetic elements from Archaea and Bacteria will help us understand the dynamic genetic network of the microbial communities in the deep biosphere.

Keywords: Deep sea hydrothermal vents, Archaea, thermophilic Bacteria, Virus.

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Design and optimisation of phase variation-based biosensors capable of highly sensitive bacteriophage detection as well as phage receptor discrimination.

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Environmental monitoring of bacteria using phage-based biosensors has been widely developed for many different species. On the other hand, there are only a few available methods to detect specific bacteriophages in raw environmental samples. This is of great importance not only to isolate new bacteriophages directed to kill a given bacterial species, but also to monitor bacteriophage presence in industrial processes or to control the fate of bacteriophage population during and after therapeutic treatment. In this work, we developed a simple and very efficient assay to rapidly monitor the phage content of a given sample. The present assay is based on the bistable expression of the *Salmonella enterica opvAB* operon. Under regular growth conditions *opvAB* is barely expressed and bacteria harbour long chained-LPS. However, in the presence of phages that use LPS as a receptor, the population expressing *opvAB* is selected as this operon confers short chained-LPS providing phage resistance. Therefore, using an *opvAB::gfp* fusion we could monitor with a high sensitivity LPS-binding phage in various media, including raw water samples. To enlarge our phage-biosensor panoply, we also developed several coliphage biosensors that proved efficient to detect LPS- as well as protein-binding coliphages. Moreover, the combination of these tools allows to identify what is the bacterial receptor triggering phage infection. The *opvAB*::*gfp* biosensor thus comes in different flavours to efficiently detect a wide range of bacteriophages and decipher the type of receptor they recognize.

Comparative genomics of Xanthomonas oryzae pv. oryzae bacteriophages

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Xanthomonas oryzae pv. *oryzae*(*Xoo*) is the causative agent of bacterial leaf blight (BLB) of rice. BLB of rice is a devastating disease causing severe economic losses, especially on Asia and western Africa. Bacteriophages may provide an environmentally friendly, effective solution against *Xoo*. Isolated *Xoo*bacteriophages were grouped into two major groups: OP1- and OP2-likge phages (Wakimoto et al 1960).
In our laboratory – Enviroinvest Corp. in Hungary – ten different OP2-like phages were isolated from Vietnam and from Philippines previously, they were characterized and their complete genomes were determined (Kovács et al 2019). As a result of our cooperation with Ralf Koebnik's laboratory in the IRD Montpellier, two more bacteriophages were isolated against *Xoo*. These new phages were characterized and sequenced as well. Based on terminase large subunit encoding gene sequences, OP1- and OP2-like bacteriophages build a distinct phylogenetic group and OP2-like phages could be further divided into 2 major groups. Comparative full genomic analysis of the 12 newly isolated OP2-like bacteriophages revealed the phylogenetic relationship among the different isolates. A 322 bp conserved intergenic region in the OP2-like phage genomes were discovered when genome sequences of the 12 novel OP2-like phages were compared to OP2's genome. Analyzing mutation frequencies along the OP2-like phage genomes enabled us to track steps of the molecular evolution of OP2-like bacteriophages. Host-specificity of these newly isolated bacteriophages were tested on representative strains of *X. oryzae*(*Xoo* and *Xoc* from Asia and Africa, on strains of *X. oryzae* from the USA and on *X. oryzae* isolated from southern cutgrass). All of the examined phages (two from Vietnam, two from Philippines and two from Montpellier) could infect only *X.oryzae*pv. *oryzae*strains from Asia, contrarily, these phages were produced no effect against *Xoo*strains from Africa and against *X. oryzae*pv. *citri*strains.
Our study provides data on the genomic characteristics, phylogenetic relationships and a route of molecular evolution of mostly novel *Xoo*OP2-like bacteriophages.

Traitement de sauvetage d'un empyème extradural par phagothérapie personnalisée en association avec antibiothérapie intraveineuse.

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Contexte: La complexité d'une infection bactérienne peut être liée aux résistances aux antibiotiques (ATB) mais aussi à d'autres facteurs (matériel étranger, site infectieux, terrain). Après des décennies d'oubli, les bactériophages (Bϕ) reprennent une place dans l'arsenal thérapeutique des infectiologues. Nous rapportons un cas d'empyème extradural (ED) à *S. aureus* sensible à la méthiciline (SAMS), nécessitant un traitement de sauvetage (TS) associant de la dalbavancine (DAL) intraveineuse et un cocktail de Bϕ personnalisé.
Matériels / méthodes: Les Bϕ ont été produits par Pherecydes-Pharma®Lab's. La souche de SAMS était sensible à deux des Bϕ testés. L'injection de Bϕ a été réalisée après accord écrit de la patiente et approbation du caractère compassionnel par l'Agence nationale de sécurité nationale du médicament (ANSM).
Résultats: Une patiente de 29 ans atteinte de neurofibromatose de type 1 a été pris en charge fin 2018 pour un empyème ED. L'histoire chirurgicale débute en 2007 par l'excision d'un astrocytome pilocytique. Le suivi sera émaillé de plus de 10 neurochirurgies conduisant à réaliser deux cranioplasties (CP) en 2015 puis 2018. Les 2 CP se compliqueront d'empyème ED Après l'ablation de la deuxième CP un lambeau libre musculo-cutané de recouvrement a été réalisé qui finalement se nécrosera 4 mois plus tard (Juillet 2018) avec empyème à SAMS. L'écoulement a persisté par la fistule cutanée malgré de nombreuses lignes d'ATB adaptées. L'état neurologique de la patiente s'est lentement dégradé du fait d'un pneumatocele comprimant les deux lobes frontaux. Un TS par DAL IV 1500 mg à J1 et J15 associé avec l'instillation locale d'un cocktail de 2 Bϕ par la fistule cutanée a été réalisé. L'instillation a été réalisée 3 jours après la première dose de DAL IV. L'écoulement purulent s'est tarit et aucun effet secondaire n'a été observé. Un nouveau lambeau de recouvrement a été réalisé. Les prélèvements per opératoires sont restés négatifs. À 9 mois de ce traitement la patiente est en vie, la cicatrisation du Lambeau est parfaite et la patiente est revenue à son état neurologique de base. Elle est maintenant retournée à son domicile.
Conclusions: Nous présentons le premier cas d'utilisation d'une association phagothérapie personnalisée et dalbavancine IV dans le contexte d'empyème extra dural à SAMS complexe. Ce résultat ouvre de nouvelles perspectives dans la gestion des infections complexes.

« Phagothérapie - Retour d'expérience et perspectives » - Compte-rendu du CSST de l'ANSM

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Par rapport à l'année 2016 où un premier comité scientifique spécialisé temporaire (CSST) de l'Agence nationale de sécurité du médicament et des produits de santé (ANSM) intitulé « Phagothérapie » s'était réuni, ce second CSST (21 mars 2019) a permis un retour d'expériences sur des administrations compassionnelles de bactériophages au niveau hospitalier en France. Il est admis que la dizaine de patients traités à ce jour correspond à des situations particulièrement complexes en pratique clinique (absence d'alternative appropriée, terrain débilité, pronostic fonctionnel voire vital engagé). En majorité, les infections traitées étaient des infections ostéo-articulaires, étaient dues à Pseudomonas aeruginosa et Staphylococcus aureus et ont fait l'objet d'une administration locale in situ des bactériophages. Le CSST a également permis un partage d'informations sur l'avancée de recherches et de projets d'études.
Plusieurs enjeux de la phagothérapie ont été discutés au cours de ce CSST, en particulier :
- Difficultés particulières de trouver des phages actifs, disponibles et de qualité compatible à l'usage en clinique, au regard des lieux limités de production pour lesquels on dispose de garanties au plan qualité, de l'absence d'une phagothèque en France et du nombre limité des types de phages produits,
- Des potentielles difficultés d'interprétation de l'activité des phages en vue de leur sélection pour administration,
- De l'aspect critique des modalités d'administration en termes d'impact sur l'activité des phages,
- De la nécessité de lutter contre la désinformation des patients
L'ensemble des enjeux soulevés par la phagothérapie a conduit à plaider en faveur de la mise en place d'une plateforme nationale d'orientation et de validation du recours aux phages pour encadrer le recours aux bactériophages en France et qui pourrait à terme travailler à la mise en œuvre d'une production académique en France de phages pour usage clinique à partir d'une phagothèque. Au vu des enjeux critiques pressentis, il est attendu que cette plateforme soit mise en place à un niveau ministériel avec les instances impliquées dans l'organisation des soins.

Bacteriophage genome transfer mechanisms at the Gram-negative cell wall

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At the Gram-negative cell wall, tailed bacteriophages encounter a variety of biomolecular structures. These contacts serve in receptor recognition, bacteriophage particle positioning on the outer membrane and initiate DNA release from the capsid and formation of transmembrane structures for genome transfer. The molecular mechanisms of these steps are not understood on a molecular level despite the high prevalence of tailed phages isolated against Gram-negative pathogens for the purpose of developing new antimicrobials. To elucidate mechanisms of initial rearrangement steps in contact with the Gram-negative cell envelope we have analyzed *Salmonella* (S.) and *E. coli* phages from the three tailed morphology types with short (*Podoviridae*), long, non-contractile (*Siphoviridae*) or contractile tails (*Myoviridae*).
In order to explore the biochemistry of cell surface adsorption and phage particle opening, we use simplified models of the Gram-negative membrane. By elucidating the function of the bacteriophage molecular infection machine under defined conditions *in vitro* we might thus describe conditions for successful infection events *in vivo*. We study O-antigen specific phages that exclusively release their DNA upon contact and enzymatic action on lipopolysaccharide (LPS) receptors (1,2). Using fluorescence spectroscopy, we have studied the timing of genome ejection in the different tail morphologies and the role of the O-polysaccharide receptor upon contact with pure LPS (3,4). We found that the tail morphology dictated the velocity by which the phages ejected their DNA, however, DNA release kinetics were not dependent on the carbohydrate composition of the O-antigen receptor (4,5). Triggering phage particle opening *in vitro* with more complex receptor systems like outer membrane vesicles moreover showed the influence of membrane composition and vesicle lumen content on genome ejection. DNA release only occurred at permissive temperatures *in vitro*. Whereas a contractile-tailed myovirus had O-antigen adsorption coupled with particle opening, short-tailed podoviruses stayed adsorbed to their host O-antigen receptor without genome loss at low temperature. We therefore propose that bacteriophages differentially exploit the bacterial host cell envelope for infection under a given environmental condition.

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Structural study of the tail tip of siphophage T5

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The vast majority (96%) of bacteriophages - bacterial viruses - possess a tail that allows host recognition, cell wall perforation and safe viral DNA channelling from the capsid to the cytoplasm of the bacterium. The majority of tailed phages, the *Siphoviridae*, bear a long flexible tail formed of stacked tail tube proteins (TTP) that polymerise around and along the tape measure protein (TMP). At the distal end of the tail, the tail tip complex harbours the receptor binding protein·s (RBP). Interaction between the RBP with the host surface triggers cell wall perforation and DNA ejection, but little is known on these mechanisms for *Siphoviridae*. We aim at characterising the mechanism of cell wall perforation and DAN ejection in the siphocoliphage T5. We have determined the structure of T5 tail tube, before and after interaction with its *E. coli* receptor, and shown that host binding information is not propagated to the capsid by the tail tube [1]. We now focus on the structure of the tail tip complex, and will present preliminary data on the structure at 3.5 Å of the tail tip complex, determined by cryo-electron microscopy and single particle reconstruction on purified T5 tails. We could trace 4 of the 6 proteins that compose it [2, 3], and we will discuss the structural homology with other phages and phage-derived complexes.

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Functional and structural study of theT5 bacteriophage immunity protein Llp

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The phage infection is triggered by host recognition thanks to the Receptor Binding Protein binding to its receptor at the surface of the cell. This interaction allows the viral DNA to be delivered into the host cytoplasm. This first step of infection is followed by viral replication and eventually liberation of the new virions. During this vulnerable time, phages protect the new viral factory from over-infection. In coliphage T5 this protection is mediated by a periplasmic lipoprotein, Llp, targeted to the inner leaflet of the outer-membrane, that binds the phage receptor FhuA. Llp main biological function is probably also to prevent the inactivation of progeny phage by active receptors present in outer-membranes debris of lysed cells, and increasing their chance of infecting a new host.
For phage T5, the Receptor Binding Protein pb5, the host receptor, FhuA and the "immunity protein", Llp, have been identified and are biochemically available. We thus aim to decipher the mechanisms of host recognition and its inhibition at the molecular level.
During my PhD I will study the interaction between Llp and FhuA, to explain the inhibition of phage RBPs binding to FhuA. I have already obtained clones of bacteria expressing Llp in acylated and soluble forms (*i.e.* without the N-terminal acylated Cysteine). I performed a first purification that lead to 0.4 mg of pure and soluble protein. I have also started to analyse the overexpressed acylated Llp localisation in bacterial membranes.

The cost of immigration control and the benefits of illegal immigration

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The discovery of immigration control opened up the floodgates for molecular biology over half a century ago. However, despite decades of intense research, no one has asked whether immigration control carries a cost to the host. We show that at the level of the individual, the host incurs a cost by employing immigration control. Surprisingly, immigration control can also be used for illegal immigration purposes that benefit the host. The cast of this work consists of several classic characters: EcoRI & EcoRV - immigration control , phage lambda - the immigrant, and *E. coli* - the host.

Phage-associated modifications of bacterial lifecycle

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Phage-bacteria symbioses span over a wide range of interactions that mediate each other's survival and evolution. While the importance of phages in driving bacterial demography has received considerable attention, we still know very little about whether and how phages modify bacterial lifecycle. Here, we addressed this question using *Myxococcus xanthus* as experimental model. This Gram-negative bacterium displays a fascinating lifecycle alternating between metabolically-active vegetative cells and dormant spores. Upon starvation, vegetative cells aggregate and develop into multicellular structures called fruiting bodies. Within fruiting bodies, some cells transform into spores that can resist a broad spectrum of harsh environmental conditions while retaining the ability to probe their surroundings. The spores can therefore leave dormancy and germinate when conditions are favourable. Crucially, phages can infect *M. xanthus* as vegetative cells and up to the onset of sporulation, in which case phage DNA may be trapped in the spores until germination. We specifically examine whether and how phages modify fruiting body formation and spore germination. We found that germination is delayed in the presence of a virulent phage, with about 50% fewer germinating cells after five hours. In contrast, the timing of germination was not affected by the two temperate phages. Still ongoing experiments are evaluating the potential for phages to both induce and modify multicellular development. We are assessing in particular the effects of phages on developmental timing, on fruiting body phenotypes and spatial organization, and on sporulation success.

Characterization of *E. coli* LF82 prophages and study of their impact on the macrophage-induced persistence of their host

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Escherichia coli LF82 is an AIEC (Adherent-invasive *Escherichia coli*) frequently found in gut microbiota from patients with Crohn's disease, a chronic inflammatory pathology of the digestive tract characterized by abdominal pains, diarrheas and vomiting. *E. coli* LF82 is able to cross the intestinal epithelial barrier and invade the underlying tissues where macrophages are normally the first line of defense against microorganisms from the lumen of the digestive tract. However, *E. coli* LF82 is able to survive inside macrophages, multiply itself and produce a lot of persistent bacteria [1]. Persisters are phenotypic variants which can survive to antibiotics and then resume normal growth once this treatment is interrupted.

In order to see if prophages could be involved in the physiology of LF82, we started the characterization of the five prophages identified in this strain [2]. We shew that one of them, a P22 homologue called Gally, is produced in high quantity in *E. coli* LF82 culture supernatants (about 108 phages/mL). However, after many attempts, its isolation is still a failure, in such a way that we suspect it to be defective. Nevertheless, Gally seems to be domesticated by its host genome. Indeed, this prophage is strongly associated with *E. coli* strains from Crohn's disease patients [3].

Focusing on the Gally prophage, we observed that some of its genes seem to be important for bacteria *E. coli* LF82 surviving endocytosis by macrophages (TnSeq, O. Espéli, unpublished). Furthermore, macrophage infection experiments suggested that Gally could also have a positive impact on the macrophage-induced persistence of LF82 strain. These last data were unexpected because they go against reports showing for two lysogens (of *E. coli* and *S. aureus*) that prophage induction reduces the population of bacterial persisters [4,5]. In LF82, we suspect that three Gally genes, *recT, mu-gam* and *abc1,* which are overexpressed when *E. coli* LF82 is endocytosed by macrophages, provide a gain-of-function to the bacterial host. Indeed, their inactivation reduces the population of LF82 persisters in macrophages. These genes could be involved in an original recombination mechanism, associating both homologous and non-homologous recombination proteins and promoting DNA repair in slow-dividing cells. Therefore, future work involves i) identification of the biochemical activities of RecT, Mu-gam and Abc1 proteins and characterization of the original phage recombination mechanism in which they may be implied, ii) understanding of the positive effect of Gally on the survival and/or macrophage-induced persistence of *E. coli* LF82.

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Lysogeny in the Lactic Acid Bacterium Oenococcus oeni Is Responsible for Modified Colony Morphology on Red Grape Juice Agar

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Oenococcus oeni is the lactic acid bacterium (LAB) that most commonly drives the malolactic fermentation (MLF) of wines and ciders. Because spontaneous MLF conducted by indigenous LAB strains can be capricious malolactic starters are developed and available for sale, but did not ensure the success of MLF [1]. Prophages prevalence in *O. oeni* genomes is high, although its technological interest, roles of lysogeny on bacterial fitness and population dynamics still poorly understood and studied [2,3,4].

Here we present a rapid and inexpensive method that allows the differentiation of lysogenic derivatives of *O. oeni* on red grape juice (RGJ) agar medium, simplifying the construction of isogenic pairs of strains that carry or not prophages for further characterization.

When turbid plaques formed by temperate phages on a sensitive strain lawn are picked and streaked on RGJ plates white and red colonies appear after incubation. Prophages specific PCR reactions showed that red CFUs were lysogenized while white not.

It was demonstrated that this phenotype change is linked to prophages spontaneous induction within the intracolonial population as exogenously provided purely lytic phages also causes the white to red colony color switch.

Experiments on white grape juice agar medium supplemented or not with polyphenolic compounds showed that red color is linked to these molecules present in red grape.

We posit that intracolonial lysis due to spontaneous prophage induction promotes interactions between lysed cells material and polyphenols, conferring a red color to the lysogenic colonies.

Moreover, the method was successfully adapted to other LAB species.

The possibility of distinguishing between lysogenic and non-lysogenic strains on RGJ plates opens opportunities to easily study dynamics of lysogenization of populations.

Molecular mechanisms of selective viral DNA recognition and packaging of the bacteriophage SPP1

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Encapsidation of a double-stranded DNA genome into a preformed icosahedral procapsid is a crucial step of the viral cycle of tailed bacteriophages and herpesviruses. In these viruses, the recognition and encapsidation of viral DNA is mediated by a protein complex, the terminase. This molecular complex recognizes specifically, cleaves, and translocates the viral DNA into the procapsid through a pore formed by a portal protein. In some cases, errors occur leading to bacterial DNA encapsidation. These events lead to generalized transduction of host genes rendering the virus a vector for horizontal transfer of genetic elements. SPP1 is a model system to study the molecular mechanisms of viral DNA packaging in tailed bacteriophages. In this virus, the terminase complex formed by gp1 and gp2 recognizes the viral DNA by binding to the sequence *pac*. In order to understand how the terminase discriminates between viral and bacterial DNA, we are studying the specificity of interaction between the terminase and *pac*. Sequential deletions and substitution mutagenesis of *pac* yielded infective phages demonstrating that a large part of *pac* is dispensable. However, extensive degeneration of the *pac*R sub-region was deleterious and suppressor mutations arose. In these revertants, we observed mutations in the oligomerisation domain of gene *1* that may affect its structure. We also identified some mutations in *pac*R itself showing that a poly-A sequence is important for phage DNA recognition. The effect of deletions in *pac*L, localized upstream of the gene encoding gp1, was compensated by mutations that led to overproduction of the terminase complex. The data show that increasing terminase concentration ensures correct *pac* recognition and cleavage in spite of elimination of the *pac*L site for gp1 binding region while degeneration of the other binding site, *pac*R, can be compensated by changes in the gp1 protein.

Bacteriophage T5 early genes: a model to dissect first host-phage interactions.

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T5 is a lytic bacteriophage infecting *Escherichia coli*. It delivers its DNA in two steps: first, 8% of the genome enters the cell and the injection pauses. Early viral genes are expressed, the gene products inactivate host defenses and trigger a massive host genome degradation; in a second step, the viral DNA delivery resumes allowing the phage to complete its infection cycle. Due to this two-step infection mechanism, only the early genes are exposed and expressed in the cytoplasm after the first step. Therefore, T5 constitutes an excellent model to study the early events in host cell takeover.

A sequence analysis of the phage DNA delivered in the first step reveals 17 open reading frames organized in three transcriptional units. So far, two genes were shown to be essential for T5 infection: *A1* encodes a DNase and *A2* a putative transcription factor. The aim of this study is to elucidate the function of the other 15 genes in infection.

Through genetic engineering, we obtained phages carrying single- or multiple-gene deletions, indicating that twelve early genes are dispensable for infection. However, the phage deleted in *dmp*, a conserved gene among the genus *Tequintavirus* encoding a deoxynucleoside monophosphatase showed a delayed eclipse and latent times, and its burst size is 25% lower than that of the wild type. On the other hand, while mutants in the genes *02*, *05*, and *07*, display a subtle delay only in their latent times, they unexpectedly release ca. 70% more phages than the wild type. Such differences point to distinct roles for these genes and the fitness cost linked to their deletion might increase in the phage's natural environment.

In a parallel study, we analyzed the outcome of ectopic viral gene expression in bacteria. While the expression of ten early genes had no effect on bacterial growth, we observed that overexpression of genes *05* and *08* slowed bacterial multiplication, and that of genes *A1* and *13* completely abolished it. Taken together our results suggest that at least seven of the 17 early genes control the bacterial cell host takeover.

Our results could help identify the main obstacles phages must overcome to keep the pace in the arms' race they are in with their hosts. Those obstacles could become interesting targets for antibacterial agents in the future.

AppY, a new RpoS regulator from prophage origin

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In *E. coli* K-12, the DLP12 defective prophage contains the *appY* gene, which encodes for a transcriptional regulator from the AraC family. So far, only two AppY target genes encoding the hydrogenase 1 and the cytochrome bd-II oxidase have been identified [1]. More recently, AppY has been involved in the regulation of the master regulator RpoS suggesting that AppY has other target(s) [2].
RpoS is the major sigma factor during stationary phase and under many stress conditions in γ-proteobacteria. When not needed, RpoS interacts directly with the adaptor protein RssB, which brings it to degradation by the ClpXP protease. When a stressful condition occurs, Ira proteins block this degradation pathway by titrating RssB, leading to RpoS stabilization in the cell [2].
The goal of this work is to understand how AppY regulates RpoS stability. First, we have demonstrated that the transcriptional regulator function of AppY was not required for RpoS stabilization indicating a new role for AppY in bacterial physiology. Using different approaches, we have shown that AppY interacts directly with RssB suggesting that it is a new member of the Ira family. To characterize further the interaction between AppY and RssB, we have performed a genetic screen to isolate AppY mutants still able to regulate the transcription of target genes but unable to stabilize RpoS. The characterization of these mutants has allowed us to define a region of AppY involved in its interaction with RssB.
Previous studies have shown that upon lytic phage infection, some virulent phage-encoded effectors negatively regulate RpoS to take the control of the host [3,4]. Our work shows for the first time that AppY a protein from prophage origin, stabilizes RpoS likely allowing a better resistance to the host under stressful conditions. It thus seems that RpoS is a target of choice to optimize phage's fitness under both lytic and lysogenic infection.

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Mechanisms of replication for an efficient multiplication of bacteriophage SPP1 DNA during viral infection

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During the co-evolution of viruses and cells, viruses exploited numerous ways to hijack cell machineries for their optimal multiplication and dissemination. We study the well-characterized model tailed bacteriophage SPP1 that infects the Gram-positive bacterium *Bacillus subtilis*. This study focus on the spatio-temporal program of its genome replication during infection. Previous results showed that SPP1 DNA localizes in a defined replication *focus* in the cytoplasm [1]. Here, we describe viral and bacterial actors involved in this well-regulated process.

First, we quantified total viral DNA copies during phage infection. Almost 500 copies of the viral genome, equivalent to ~ 5-fold the total bacterial DNA mass, are synthesized in the first 25 minutes after SPP1 infection. This replication efficiency requires fast recruitment of most of the bacterial replisome proteins. It is orchestrated by the SPP1 helicase gp40 known to bind to the DnaG primase and to DnaX, a subunit of DNA polymerase III [2, 3]. Their accumulation in viral replication factories few minutes after infection suggests that the host replisome machinery is massively re-directed for SPP1 DNA replication.

After a few cycles of phage circular DNA replication (theta replication mode), there is switch generating a linear template for replication (sigma replication mode) that is essential to generate concatemeric DNA which is the substrate for encapsidation. Two proteins, the recombinase gp35 and its partner the 5'-3' exonuclease gp34.1, play a central role in this step [4]. The two proteins co-localize with the replication *focus* supporting this assignment.

Phage SPP1 implements a complex and well-regulated replication mechanism to optimize the synthesis of a large amount of viral DNA copies within a short time frame. Intriguingly, only ~ 30 % of this DNA is packaged in viral particles at 30 minutes post-infection when host lysis initiates.

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Viral Host Range database (VHRdb), an online resource to collect, browse and analyse the host range of viruses

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Viruses are ubiquitous and often infect more than one host. The range of hosts that a virus can infect to produce progenies is an information not readily available outside experts. With the massive amount of genomic data that is currently produced by large sequencing projects, many novel viruses remain to be characterized, including their host range. Host range experiments are performed routinely in many laboratories, but the results are rarely published under an exploitable format easily accessible to the community. In particular, hundreds, if not thousands of host range tests are currently performed when characterizing bacteriophages for their use as therapeutics.

The viral host range database (VHRdb) is an online resource that centralizes experimental data related to the host range of viruses. While it was initiated from data obtained from bacteriophages and bacteria, its design is compatible with viruses infecting all living forms. Users can upload their own data, with the option to keep it private or make it public, analyze results across independent sets of data, generate and visualize outputs. Data implemented in the VHRdb are linked to users and, if available, to publications and sequence identifiers. This way, users can browse publicly available data to find which host is infected by a virus, and vice versa.

The VHRdb represents a unique resource for the community to rapidly find or disseminate the range of hosts suitable for a virus, an information that can have broad interest for educational, scientific, medical and applied purposes.

If this abstract is selected for a presentation, a live demonstration of the main functionalities of the VHRdb will be performed.

Presentation of the EuroXanth COST action – planning a COST action on bacteriophage research and application

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COST (European Cooperation in Science and Technology – see www.cost.eu), is a unique platform where European researchers can jointly develop their ideas and initiatives across all scientific disciplines through the trans-European networking of nationally-funded research. It is funded by the European Union.

Bacteria of the family *Xanthomonadaceae*, including species of *Xanthomonas* and *Xylella fastidiosa*, belong to the most devastating plant pathogens continually challenging food security. Euroxanth addresses several key aspects of the pathogen-vector-host interactions from the cellular to the population level. A better insight into population structures and virulence mechanisms of the pathogens, together with the exploration of the molecular mechanisms underlying disease resistance to the pathogen, enables the development of durably resistant plant cultivars and exploitation of bio-control schemes, including the application of bacteriophages.

Based on the experiences originating from the EuroXanth, we propose a novel COST action dealing with important aspects of bacteriophage basic and applied science to integrate the knowledge on bacteriophages in order to coordinate the research on these viruses which can strengthen the application of these antibacterial agents in the EU. This presentation would provide an opportunity for discussion on preparation of the planned COST action.

POSTERS

Ecology & Evolution

A molecular ecology approach using Stable Isotope Probing and metagenomics to study viruses of methanogens' diversity

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Viruses of microbes are ubiquitous and they are major drivers of ecosystem functioning [1]. Viral ecology is rapidly developing, driven by the advent of high throughput sequencing [2]. Nevertheless, the viral diversity of certain ecosystems is still poorly characterized, such as those which we are studying in an applied perspective, the anaerobic digestion (AD) plants. AD is an environmental biotechnology which produces methane-rich biogas, a renewable energy source. It relies on the catalytic activity of complex microbial communities. To optimize AD processes, the links between operational parameters, microbial community properties and process performance must be finely understood. Indigenous viruses should thus also be considered, as they most certainly influence the matter fluxes during AD. To our knowledge, a unique publication has been dedicated to AD metavirome sequencing [3]; it highlights their specific diversity compared to metaviromes from other types of ecosystems.

Our study focuses on the *in situ* diversity of DNA viruses of methanogenic archaea, the latter being key players of AD. We are developing an original approach based on stable isotope probing (SIP) [4] and high throughput sequencing. SIP consists in separating DNA molecules according to their density by ultracentrifugation on a CsCl gradient. Using various 13C-labeled methanogenesis substrates enables us to activate selectively distinct populations of methanogens and to enrich them in 13C [5]. The viruses that infects these methanogens should logically also be labelled with 13C, so that it should be possible to separate their DNA by SIP before sequencing them with a shotgun method.

We first proved the concept of SIP application to viral DNA on a pure strain model. We showed by SIP the incorporation of 13C into T4 bacteriophage DNA, obtained by farming on *Escherichia coli* cells grown in minimal medium, containing either unlabeled or 13C-glucose as sole carbon source. A range of 13C/unlabeled glucose ratios (20% to 80%) was also employed to show that T4 DNA 13C-enrichment is proportional to the 13C glucose rate of the medium.

For a first application to AD communities, we selected formate as unique carbon source, expecting to activate Methanobacteriales members as main methanogens. At 3 distinct incubation time points, we prepared cell and virion pellets, extracted their DNA separately, and generated their SIP profiles. For the cellular DNA SIP fractions, we are currently determining the 16S metabarcoding profiles. Viral particles were observed by electron microscopy. In addition to cosmopolitan head-tail virions, morphotypes typical of archaeal viruses were interestingly present, such as spindle shapes (*Fuselloviridae, Salterprovirus*) [6].

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Detection of an archaeal-specific viral family, previously thought to infect only hyperthermophiles, in human gut metaviromes

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Viruses of archaea have an exceptional diversity [1]. Several of their families are shared with bacteria, typically those from the cosmopolitan Caudovirales order. However, the majority of them are archaea-specific; they have very diverse genomic contents and morphotypes, such as two-tailed (Bicaudaviridae), spindle (Fuselloviridae, Salterprovirus), rod (Rudiviridae), or filamentous (Lipothrixviridae) shapes. Most of the known archaea-specific viruses infect hyperthermophiles and halophiles. Nevertheless, the viruses of many archaeal phylogenetic groups remain poorly explored, such as those of methanogens. Six of the seven known methanogen-infecting viruses belong to the Caudovirales order [1]. Interestingly, a spherical virus infecting *Methanosarcina* strains has recently been isolated [2]. Named MetSV, it probably represents a new family [1]. The smacoviruses have moreover been suggested to infect methanogens rather than human, based on metavirome analysis [3]. Finally, unusual morphotypes have been detected in metaviromes from anaerobic digestion plants, as well as contigs of unknown hosts from various archaea-specific viral families: Salterprovirus, Bicaudaviridae, Fuselloviridae, Lipothrixviridae, and Rudiviridae [4].

To identify putative viruses of methanogens in metaviromes from anaerobic ecosystems, we developed a bioinformatics pipe-line based on metaSPADES [5] for assembly and Kaiju [6] for taxonomic assignation. As a first test, we applied our pipe-line to the raw reads of 4 previously published human gut metaviromes [7]. A total of 12 short contigs (~1 kb-3.5 kb) were assigned by Kaiju to known families of archaeal viruses, corresponding to Rudiviridae exclusively; this assignation was confirmed by blast analyses. Using WiSH [8], we predicted their possible hosts among archaea. Nine of them had best predictions for Sulfolobales members, their presently known acidothermophilic hosts, while the remaining 3 were predicted to infect methanogens (genera *Methanosarcina*, *Methanosphaera* and *Methanobrevibacter*). It suggests that some of these contigs could originate from viruses of methanogens, consistent with methanogens being the dominant archaea in the human gut.

In the future, we plan to include a CRISPR-spacer matching tool to our pipe-line, to predict the hosts by a complementary method. Using a state-of-the art assembler [9] yielded only short Rudiviridae contigs, likely because ruvidiruses have a low abundance in the human gut. It advocates for the use of enrichment approaches to study them more specifically. Our results reinforce the notion that archaea-specific viruses may not be mostly restricted to extremophiles and could be more widespread in the biosphere than previously thought.

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Genome analysis of Pseudomonas phage PPA2

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Introduction One of the most important complication of diabetes mellitus is diabetic foot wounds caused by different bacteria. In our study, genome analysis of the phage PPA2 isolated from fecal matter, active against *P. aeruginosa* strains of diabetic foot origin was sequenced. **Material and Method**: To isolate the phage DNA, filtered phage samples were centrifuged at 15000xg for 2 hours at 4 °C. Then supernatant was discarded and pellet was dissolved in Tris-EDTA buffer with RNase, proteinase K was added and tubes were incubated at 56 °C for 1 hours. DNA isolation was completed with phenol-chloroform-isoamyl alcohol precipitation. Phage DNA sample was digested with EcoR1 enzyme and visualized by gel electrophoresis. The phage genome was sequenced using the Illumina MiSeq system at the Eurofins Genomic (Germany). **Results:** The phage genome was sequenced using the Illumina MiSeq system at the Eurofins Genomic (Germany). Phage PPA-2 has a genome of 87,845 bp, with a coding percentage of 91,48% and a G+C content of 54.77 %. In a dot plot alignment, the PPA-2 genome showed similarity and 97,75% overall nucleotide identity to Pseudomonas phage PAK_P3 (GenBank accession no. KC862299.1). When compared with gene bank following homologies with known phages were found: 97,75 % identity with PAK_P3 phage, 97,74% with P3_CHA-2, 97,74% with PAK_P5, 97,38% with KPP10 and 96,76% with vB_PaeM_G1 phages. We predicted 162 unique coding sequences, of which 35 were assigned a predicted function and 127 are hypothetical. We identified genes for DNA replication, including a DNA ligase, a DNA helicase, a DNA polymerase, a putative primase/helicase protein, and the putative terminase large subunit. Furthermore, we identified genes that encode proteins for DNA and nucleotide metabolism, such as a ribonucleotide reductase, a RNA ligase and a tRNA-Tyr. The gene for host-cell lysis encode for an cell wall hydrolase. The genes for morphogenesis encode for a major capsid protein and tail fiber proteins. **Conclusion**. It is important to isolate new phages active against diabetic foot infection agents. Phage PPA2 have the potential to be used in phage treatment of diabetic foot infections due to *Pseudomonas*.

Proline and arginine metabolism at the interface of stationary phase physiology and bacteriophage infection in Bacillus subtilis

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Nutrients are often scarce in natural environments and nutrient starvation induces very different physiological and metabolic states in bacteria than those found in their exponentially growing counterparts. However, the vast majority of bacteriophage infection studies are performed with exponentially growing bacteria. As bacteriophages depend on host metabolism in order to effect viral replication, it is perhaps not surprising that infection efficiency of stationary phase cells in *B. subtilis* and other bacteria is drastically reduced. Though this is known, there is a general paucity of literature on this subject and little is known of the mechanisms behind this phenomenon. Previous work from our lab demonstrated the requirement for PutB, an L-Proline dehydrogenase, for post-exponential phase infection of *B. subtilis* by bacteriophage SPP1 [1]. L-Proline may be exploited as a sole source of carbon and nitrogen by *B. subtilis* and its catabolism converges with that of L-Arginine in production of γ-Glutamate-5-semialdehyde, a precursor to L-Glutamate. Expression of genes involved in the uptake and catabolysis of these amino acids is also upregulated during stationary phase and in sporulation [2,3]. Here we begin to explore the roles of proline and arginine catabolysis in infection of *B. subtilis* by two bacteriophages, SPP1 and SPO1, and in the post-exponential growth phenotypes of *B. subtilis*. Initial results show that disruption of L-Proline and L-Arginine catabolysis at different stages has effects on bacterial viability during stationary phase and on spore formation. Furthermore, SPP1 and SPO1 plaque morphology and ontology is affected by perturbing these pathways. Adsorption of SPP1 to the bacterial cell surface and SPP1-mediated bacterial lysis are also affected. Together, these results suggest important roles for L-Proline and L-Arginine catabolysis in survival strategies of stationary phase *B. subtilis* cells and that these metabolic pathways may act as more general gatekeepers of *B. subtilis* susceptibility to bacteriophage infection.

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THERAPY AND BIOTECHNOLOGY APPLICATIONS

DNA-free POETential, a synthetic biology project for the iGEM competition: Repurposing a DNA-less bacterium into an “RNA cell” with a little help from phages

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The purpose of many teams participating in the international Genetically Engineered Machine competition is to design genetically modified organisms (GMOs) with new interesting properties. To address the issue of GMO containment, many teams have engineered so called kill-switches based on toxins that can be expressed under controlled conditions. Since 2008, at least seven teams have attempted to use deoxyribonucleases to eliminate the genome of their reprogrammed machine, in order to destroy both the GMOs and their DNA and thereby prevent possible horizontal gene transfer. However, all teams were, to our knowledge, unsuccessful.

Our team GO Paris-Saclay decided the challenge was worth the effort, because DNA degradation does not mean immediate cell lysis, and a DNA-less cell could still perform preprogrammed functions. Actually, one published report showed that *Bacillus subtilis* cells are metabolically active for up to 5 hours following complete degradation of DNA (Elbaz and Ben-Yehuda, 2015, *mBio*). Using an arabinose-inducible promoter that could be tightly repressed by glucose, our team successfully cloned in *Escherichia coli* three distinct nucleases from different phages. Within 30 min of induction, bacterial cell growth was arrested and CFU recovery was reduced 1000- to 10000-fold. Bacterial DNA degradation was confirmed by the decrease in genomic DNA recovery and by the absence of DAPI staining observed under fluorescence microscopy.

As a next step in functionalizing our DNA-free-cells, we observed that cells producing one of the nucleases along with methotrexate-degrading enzymes were able to decrease the concentration of this toxic anti-cancer drug. This result suggests that our DNA-free cell could be used in bioremediation. In another attempt to repurpose cells devoid of DNA, we infected them with the RNA phage MS2 and could observe a 100- to 1000-fold increase in phage titer, indicating that the phage RNA was able to replicate within the cells producing nucleases. These results suggest that our DNA-less cells might have transiently resembled cells that once thrived in the “RNA world”, i.e. cells where the replicating genetic information was carried by RNA instead of DNA.

Taken together, our work opens interesting avenues in developing new kinds of synthetic organisms devoid of DNA. These possibilities, both exciting and unsettling in a world where DNA is a synonym for identity, were the object of an artistic exchange with scientists and non-scientists, under the form of Japanese-style poems.

Bacteriophages active against Meticillin Resistant Staphylococci isolated from bovine mastitis infections.

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Mastitis is inflammation of breast or udder and generally associated with microbial infection. *Staphylococcus aureus* is one of the most important bacteria that causes of 19.97%-65% of cases. More than 38% of total health budget in milk facilities is spent only for treatment of mastitis infection. Phage therapy is emerged as alternative treatment way for infection especially for cases those caused by antibiotic resistant bacteria. In this study, 10 phages isolated from different samples in our laboratory were tested against various staphylococci strains. **Material and Method.** Samples from water pools for animals, waterbath in the laboratory and faecal matter were collected to use as phage sources. Water samples (50 mL) and solid samples diluted in sterile distilled water (%2 v/v) and after centrifugation at 5000xg for 15 minutes, supernatants were collected and filtered by membrane filter with 0.22 mm por size. Double agar layer technique was used to isolation and purification of phages. Efficiency of phage suspensions were tested against Methicillin resistant *Staphylococcus aureus* strains (n=7), and totally 5 coagulase negative staphylococci (*S. epidermidis, S. haemolyticus, S. hominis, S. cohnii and S. caprae*). Phage stocks were prepared and maintained at 4 °C until use. **Results.** As a result of the study PSA-1, PSA-3, PSA-6, PSA-7 and PSA-10 phages showed inhibitory effect against all bacterial strains used in the study. **Conclusion.** Phage therapy may be an important way of treatment as well as way of protection against mastitis. Among 10 phages tested 5 were active against all staphylococci tested.

Strategy using phages to control Staphylococcus aureus responsible for bovine mastitis

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1 : VETOPHAGE

Bacteriophages (phages) are very abundant in livestock. The goal of our study was to evaluate the diversity of phages in contact with cow in order to evaluate their potential for phagotherapy of mastitis. We have been able to detect and isolate phages in different samples: in water, in the digestive tract, in faeces and in raw milk. Phages were characterized by restriction enzymes and sequenced. After sequencing, the phage genome was compared to others published in the NBCI database. It is interesting to note that several phages isolated in different countries have a high percentage of identity (99%). For example, a phage isolated in France in our study exhibited a 99% identity percentage with phage K (ATCC 19685-B1-USA), with phage 812 isolated in Czech Republic and with phage HYZ21 isolated in China. But several phages isolated by Vetophage were also very different from others, exhibiting a maximum of 88% of identity on 80% of the covers, showing that they were new phages. The reduction of the number of bacteria by phages was then studied in a culture medium and in raw milk. The bactericidal activity of phages has been compared to that of antibiotics (ABs). The results showed that ABs and phages were efficient to reduce the count of bacteria in culture medium, but all ABs and phages were inhibited in raw milk. Only the use of phage cocktails could enable us to reduce the number of bacteria in raw milk. These results are promising as our phage cocktails resulting from phage diversity, and particularly from our new types of phages isolated, and active on raw milk could be used for intramammary treatment.

The use of interferometric microscopy to quantify viral particles in complex samples such as fecal filtrates

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It is now largely accepted that the intestinal microbiota plays a key role in Intestinal Bowel Diseases (IBD). An imbalance in the composition and diversity of the intestinal microbiota (i.e. dysbiosis) of patients has been repeatedly pointed out by several teams. There are also indications that phages, the viruses infecting bacteria, might play a role in this family of diseases. First, it was found that patients had higher loads of virus like particles in intestinal biopsies compared to healthy controls. Second, several metagenomics studies indicate that the intestinal virome is modified in IBD patients compared to healthy people.
In order to differentiate healthy and IBD fecal samples on the basis of their phage profiles, we want to develop a means to enumerate rapidly viral particles in fecal samples, based on interferometric microscopy. Our device, a new type of homodyne interferometer relying on interferences between a reference beam and the light scattered by phages, will permit to compare on large scales viral loads of healthy subjects and IBD patients. However, we found that fecal filtrates are largely dominated by extracellular vesicles, which are also detected by interferometry, and not easy to distinguish from viral particles. Moreover, it is complicated to quickly count phages and to purify them from vesicles with other technologies available. We therefore aim at developing a protocol allowing the destruction of extracellular vesicles, without damaging phages. The use of the interferometric microscopy has already been a real time-saver, as it enabled us to dispense with the realisation of phage plaques to verify the efficiency of the different protocols tested to purify phages from unwanted vesicles. Several of them are presented here, and these preliminary results are encouraging for our further understanding of the implication of phages in IBD, and the development of a diagnosis tool for such conditions.

Assessing phage therapy against the plant pest Xylella fastidiosa

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Associated with numerous socio-economically and ecological important plant diseases, *Xylella fastidiosa* (*Xf*) is one of the most important threats to plant health worldwide where more than 560 plant species have been listed as potential hosts. This xylem-specialized pathogen, transmitted by xylem feeding insects, belongs to the plant-associated *Xanthomonadaceae* family. So far, five different subspecies of *Xf* have been formally accepted: *fastidiosa* (*Xff*), *multiplex* (*Xfm*), *pauca* (*Xfp*), *sandyi* (*Xfs*) and *morus* (*Xfmor*). Long-known in the Americas, this plant-pathogenic Gram-negative and slow growing bacterium is emerging in Europe, probably introduced through commercial exchanges [1]. Listed as a quarantine pest, three subspecies (*Xfp, Xfm Xff* and *Xfs*) were recently detected in Europe, mostly in the Mediterranean basin, causing a variety of diseases such as the Leaf Scorch of Olive trees in Italy [2,3].

In the absence of efficient and authorized methods to control *Xf* infections, a major challenge is to develop environmentally friendly biotechnologies to control this plant disease. In this context, we propose the use of bacteriophages to control *Xf* infections as recently proposed to control destructive bacterial crop diseases. Indeed, a few phage cocktails are now available to treat diseases caused by *Xanthomonadaceaes* or *Ralstonia solanacearum* [3].

Different aspects of my work dedicated to phage therapy against *Xf* will be addressed: (i) To get rid of the difficulties linked to *Xf* culturing, I developed an indirect approach using *X. albilineans,* a fast-growing organism and close relative of *Xf* to enrich naturally occurring phages actives on *Xf*. (ii) I will report the isolation, the characterization and the host range of various virulent phages obtained from different environmental sources such as *Xf* insect vectors and more generalist sources such as raw sewage influents. (iii) Finally, I will discuss about the therapeutic and prophylactic application of two of the virulent phages I isolated as a biocontrol treatment on *Xf*-infected plants.

In our studies, *in vitro* experiments confirmed the lytic activity of two isolated phages against *Xf* with a host range spanning *Xff, Xfm*, and *Xfp* that are present in Europe. To our knowledge, this could be the first report of isolation of virulent phages with a lytic activity on European strains of *Xf*. These phages can be easily propagated on the surrogate host *X. albilineans*. The implementation of a phage-based biocontrol assay on *Xf*-infected grapevine plants indicate that bacterial growth was significantly affected in the presence of phages *in planta*. Furthermore, phage levels increased and migrated in grapevine vessels only in the presence of *Xf*. The reported phages are therefore attractive candidates for the biocontrol of *Xf* in wine industry in Europe, but also, could offer solutions to control others diseases caused by *Xf* as the olive-quick decline syndrome reported in Italy.

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Study of the prophages of Pseudomonas aeruginosa strain PP001.

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After years of extensive use, antibiotic resistance is becoming a major health concern worldwide. Alternative treatments to replace or supplement antibiotics are thoroughly researched and developed. Among them, the use of viruses that infect bacteria as antimicrobial agents, also called phage therapy, is a promising alternative. The bacteriophages used for phage therapy are virulent phages and only utilize the lytic cycle, which ends with the death of the bacteria and the release of virions in the environment. Besides virulent phages, temperate phages alternate between lytic and lysogenic cycles, where their prophage form integrates into the host bacterium's genome, and replicates through the usual prokaryotic DNA replication without killing the host. These prophages can confer to the infected bacterium virulence factors and phage immunity. They can excise themselves from the bacterial genome and enter a lytic cycle at any time. In order to produce and use phages able to kill antibiotic- resistant bacteria, it is important to work with a production strain cured of its prophages.
One of the multi-drug resistant bacteria considered as one of the most concerning is Pseudomonas aeruginosa, a gram-negative, opportunistic species mostly responsible of nosocomial infections and chronic lungs infections.
Pherecydes Pharma works on the production of virulent phages usable in phage therapy to cure Pseudomonas aeruginosa infections with standards of Good Manufacturing Practice's (GMP). In order to reach this objective, the first step of my research project is to detect and get rid of the prophages from the Pseudomonas aeruginosa strain chosen to produce virulent phages.
Pseudomonas aeruginosa strain PP001, a derivative of PAO1 is the production strain used in this study. Upon growth on Petri plates, part of the colonies streaked are often lysed and display two colony morphotypes. Moreover, culture supernatants produce phages able to form plaques on lawns of PP001. Two types of plaques with different diameters were visible, both of them clear. These results suggested there was at least two prophages in this strain, and that they were able to lyse their own lysogen, suggesting they have become superinfectious (SI), as already observed for PAO1 (Berk, R. S., J Bacteriol 86, 728–734 (1963)).
The PP001 genome was sequenced, and found to host two complete prophages from the Inoviridae family, which produce filamentous infectious particles. The first one, Pf4, also present in PAO1, is involved into the formation and dispersal of P. aeruginosa biofilms and increases its ability to adhere to surfaces, such as the lungs of cystic fibrosis patients (Rice, S. A. et al., The ISME Journal 3, 271–282 (2009).). Moreover, Pf4 is able to mutate and to lyse its own lysogenic strain becoming a SI phage (Webb, J. S. et.al, J. Bacteriol. 186, 8066–8073 (2004)). The second prophage, Pf7, and its possible effects on Pseudomonas aeruginosa are unknown at present. PP0001 mutants cured of either one or of both prophages are under construction to characterize their phenotypes.

Optical Bacterial Susceptibility test by Surface Plasmon Resonance (SPR)

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The objective of this thesis project is to develop an automated analogue of the antibiogram but for the testing of susceptibility to phages. This test will rely on Surface Plasmon Resonance (SPR) and would permit a standardization of phage therapy by providing to each patient a personalized therapy, thereby increasing the probability of successful phage therapy. In less than one hour the proposed method would specify, based on a bacterial strain isolated from a patient, which phage or phages to use to maximize therapeutic effect. More precisely, it is the ability of an array of distinct candidate phages to bind the isolated strain which is probed using SPR.

PHAGE-HOST MOLECULAR INTERACTIONS

Exploring the mechanisms of host takeover by bacteriophage T5: role of the DNA-binding protein A2

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T5 is a lytic bacteriophage that infects *Escherichia coli*. It has a unique two-step DNA injection mechanism which gives an exceptional opportunity to investigate host takeover uncoupled from the downstream phage multiplication steps. The first step transfer (FST) genes are responsible for rapid host DNA degradation and a shutoff of early phage gene transcription before the second step DNA transfer resumes (SST). Two conserved proteins, A1 and A2, were found to be essential for these early events and to control the SST. We used a multidisciplinary approach combining biochemistry, bacterial genetics and structural biology to investigate the function and structure of T5 early proteins. According to our recent findings, A1 is a Mn2+ dependent DNAse, responsible for host DNA degradation and A2 is probably a transcriptional regulator that represses the pre-early gene expression. We are currently investigating the structure and DNA -binding properties of A2, which is a dimeric two-domain protein. First NMR studies revealed the a-helical organization of the N-terminal DNA-binding domain of A2. The C-terminal dimerization domain is dynamic or in equilibrium between different conformations, which prevents its resolution by NMR. We solved this problem by analysing two protein domains separately. Finally, the solution structures of the N-terminal domain and the C-terminal domain of A2 were obtained using NMR spectroscopy. To understand the role of A2 in the transcriptional regulation of phage infection, we now want to characterize the network of the interaction of A2 with phage DNA and with other pre-early proteins. In particular, we aim at identifying the specific regulatory region of T5 DNA that is recognized by A2. The detailed characterization of A2 interactions with DNA and other proteins will be very useful to reveal its function in the host takeover.

Investigation of A2 protein partners, an essential pre-early protein of bacteriophage T5

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A century after the discovery of bacteriophages, the early steps of bacterial host takeover following phage DNA injection remain largely understudied. Thanks to their characteristics, enterophages of T5 family are privileged models to study these early events owing to their unique capability to transfer their genomes in 2 steps. During the First Step of Transfer (FST), only 8% of the T5 genome encoding the pre-early genes are injected [1]. These genes are involved in bacterial takeover resulting in host DNA degradation, inhibition of host RNA and protein synthesis and insensitivity of phage DNA to restriction. During the Second Step of Transfer (SST), the rest of the genome is injected, allowing genes involved in phage DNA replication and virions multiplication to be expressed.

In *E. coli* T5, among the 16 pre-early genes (FST genes) only 2 are essential under laboratory conditions for host takeover and resuming of SST, they encode for the A1 nuclease and the putative transcriptional regulator A2. Mutants of either *A1* or *A2* genes are not able to resume DNA transfer, thus offering the opportunity to study the early steps of host takeover independently from phage replication and multiplication processes.

The question is: how these two proteins are able to carry out all these functions by themselves? Our working hypothesis is that A1 and A2 interact with other proteins encoded by either the T5 FST and/or the bacterial genome.

Here, we present several A2 protein partners identified by Bacterial Two Hybrid (BACTH) experiment [2] using a targeted approach for the FST putative partners and a genetic screen of an *E. coli* K12 genomic library for the host-encoded partners [3].

On one hand, we found that A2 interacts only with Gp2 among 4 conserved FST proteins (Dmp, Gp2, Gp5 and Gp7). On the other hand, we identified 10 host-encoded candidate proteins involved in different cellular pathways. Five of them are transcriptional regulators all implicated in the regulation of carbohydrate metabolism. In addition, our genetic screen highlighted candidates involved in RpoS-dependent stress response, cellular division, protein translation, DNA replication and repair, and amino acid biosynthesis pathways. Regarding stress response, we confirmed that A2 interacts with IraP, an anti-adaptor protein required for stabilization of the alternative sigma factor RpoS under phosphate starvation conditions [4].

Therefore, we conclude that A2 is likely to interfere with different pathways during host takeover. The next steps will be to confirm the interaction of A2 with different candidates and to study the molecular mechanisms allowing A2 to modify the host physiology to T5 advantage.

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Filamentous phage translocation in Escherichia coli envelope does not require a functional TolQRA motor.

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Filamentous phages are non-lytic viruses that specifically infect bacteria, establishing a persistent association with their host. The phage particle parasitizes its host existing structures in order to cross the bacterial envelope and deliver its genetic material. The current model for filamentous phage infection is largely based on Ff coliphages, including fd, M13 and f1, that specifically infect *Escherichia coli* cells. Ff phages have served the development of extensive applications in genetic engineering and phage display technology. The import of filamentous phages across the bacterial periplasmic space strictly requires some of the components of a macro-complex of the envelope known as the Tol system. This complex works as a molecular motor, using the energy provided by the proton-motive force of the inner membrane to perform essential and highly energy-consuming functions of the cell, such as envelope integrity maintenance and cell division. It has been suggested that phages take advantage of pmf-driven conformational changes in the Tol system to transit across the periplasm. However, this hypothesis has not been formally tested. In order to decouple the role of the Tol system in cell physiology and during phage parasitism, we used mutations on conserved essential residues known for inactivating pmf-dependent functions of the Tol system. We identified impaired Tol complexes that remain fully efficient for filamentous phage uptake. We further demonstrate that the TolQ-TolR homologous motor, ExbB-ExbD, normally operating with the TonB protein to energize iron transporters in the outer membrane, is able to promote phage infection along with full length TolA. Together, our data provide new information on the molecular requirements controlling translocation of the filamentous phage particle in its host.

How bacteria and bacteriophage coexist in the mammalian gut?

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The composition and variations of the human intestinal microbiota has been linked to several diseases or disorders. If many studies have focused on the prominent population of intestinal bacteria, only few have highlighted the role of bacteriophages, viruses that predate bacteria. While bacteriophage/bacteria interactions have been extensively studied *in vitro*, much less is known about those in the gastrointestinal tract (GIT) of mammals.
To investigate mechanistically the role of intestinal bacteria and bacteriophages several murine models were developed over the years. Conventional mice with perturbed microbiota (by antibiotic treatments) or germ-free mice colonized by one or two bacteria of interest have been helpful but also present major limitations. More recently, a consortium of 12 bacteria representative of the five major phyla of the mouse microbiota were inoculated to germ-free mice (OMM12 mice) and were found to stably colonize the GIT of these animals for several generations, allowing the establishment of colonies in animal facilities across the world [1].
We evaluated the potential of this gnotobiotic model for studying bacteriophages/bacteria interactions in the GIT. First, we found that multiple strains of *Escherichia coli* (from both murine and human origins) can stably colonize OMM12 mice for several weeks without disturbing the relative abundance of the 12 strains. Second, we showed that the inoculation of virulent bacteriophages (either one or a cocktail of three) targeting one strain of *E. coli* colonizing the OMM12 mice led to the coexistence of similar levels of *E. coli* and bacteriophages during several weeks. When looking at gut sections, we found that phages had a weak impact of *E. coli* levels. This was not due to the rise of bacteriophage-resistant clones along the entire gut. We observed a differential spatial distribution of bacteria and bacteriophages leading to propose that the coexistence of these two antagonistic populations in the gut is in agreement with the ecological theory of source-sink dynamics.

1 - Brugiroux S. *et al.* Nat. Micr. 2016

Low efficiency of DNA mismatch repair system on lambda phage

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Viruses have very high mutation and recombination rates, even those using the replication and recombination enzymes of their cellular hosts. In particular, the mutation rate of *E. coli* λ phage is about a hundred fold higher than that of *E. coli*. Mutagenesis and recombination between diverged sequences rates depend on the error rates of replication and recombination enzymes but also on the correction of errors. In *E. coli*, correction of errors is realized by the mismatch repair system (MMR), composed of MutS, MutL, MutH and Dam proteins. Concerning λ phage, our experiments and old results suggest that its high error rates are mainly due to very bad efficiency of MMR on replicating λ DNA. We use classical genetic test and microscopic fluorescence approaches to try to identify the molecular mechanisms explaining the low efficiency of MMR on λ. We show that mismatches on λ are recognized by the MMR, but that the repair process is blocked at some latter step, by mechanisms that probably involve a saturation of MMR proteins.

ATP-dependent formation of Sak4 filaments: a first step towards the single strand annealing

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A renewed interest has focused on the study of phages due to their potential use in phage therapy. A possible drawback for their use is the rapid evolution of these entities, related to the presence in their genome of genes coding for particularly diverse and effective recombination protein that, for most of them, remain to be characterized. Sak4 from phage HK620 is a RecA-core only protein that promotes recombineering events *in vivo* (Lopes at al 2010). We recently showed that Sak4 is an ATPase that binds ssDNA in an ATP-dependent way, a characteristic that distinguishes Sak4 from RecA. Sak4 performs annealing of complementary ssDNA (SSA), albeit at a lower extent compared to RecA or the RAD52 like Redβ protein from phage λ. Remarkably, we showed that a distant homolog of the single stranded binding protein SSB, encoded by a gene present almost systematically next to the *sak4* gene, stimulates the recombineering activity of Sak4 *in vivo* as well as its ssDNA binding and its SSA *in vitro*. These stimulating effects are dependent on the last 6 amino acids of its cognate SSB, a region involved in protein-protein interactions in other bacterial SSBs. We proposed that the phage SSB facilitates the recruitment of Sak4 on DNA via a direct interaction. Sak4 of phage HK620 is the first described phage single-strand annealing protein that uses an SSB to enhance its SSA activity (Hutinet et al. 2018). Recent studies show that the Redβ protein from different phages share this ability, suggesting that SSA mediated by phage could generally depend on a recombinase/SSB duet.

However, the mechanism by which Sak4 performs SSA remains unknown. Here, we investigate the role of ATP in the reactions mediated by Sak4 and its oligomeric properties. Sak4 alone is a monomer displaying a weak ATPase activity (kcat=0.9 min-1). Presence of ssDNA stimulates this activity, however this stimulation is partly reduced in presence of its cognate SSB. Although Sak4 alone does not form distinguishable structures by transmission electron microscopy (TEM), it forms filaments in the presence of Mg2+ and ATP. Conditions allowing the binding but abolishing or decreasing the hydrolysis of the nucleotide lead to an increase of the length of Sak4 filaments and do not prevent its binding to ssDNA. Rather, these conditions seem to stabilize Sak4-ssDNA nucleofilaments observed by TEM. However, blocking the ATPase activity prevent Sak4 to perform SSA in vitro and recombineering in vivo. Altogether, these results suggest that i) Sak4 filaments and nucleofilaments are dynamics, ii) this dynamic behavior depends on ATP hydrolysis and is required to allow ssDNA annealing. Then, filament formation of Sak4 seems to be the first step in the SSA, the subsequent steps requiring desoligomerization via ATP hydrolysis. We are currently testing whether SSB influences the dynamic of Sak4 nucleofilaments via modulation of its ATPase activity.

Role of H-NS in maintenance of Gifsy prophages lysogeny in Salmonella enterica ST4/74

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The Histone-like Nucleoid-Structuring protein (H-NS) plays an essential role in DNA condensation, and in many enterobacterial gene regulation in response to different stress signals[1]. H-NS binds to DNA in a non-specific way showing preference for curved AT-rich regions preventing RNA polymerase from binding to promoter regions. Such binding has evolutionary consequences such as the silencing of horizontally transferred geneswhose non-controlled expression could be detrimental to the host[2]. The importance of bacteriophage in Horizontal Gene Transfer (HGT) is largely acknowledged[3,4]. Most *Salmonella spp*. genomes carry at least one prophage with an average of 5 prophages per genome, representing nearly 30% of the accessory genome[5]. In this context, we identified a role for H-NS in the maintenance of Gifsy-1 prophage from *Salmonella enterica* serovar Typhimurium ST4/74.

Lysogenic maintenance of Gifsy phages relies on the synthesis of a non-cleavable repressor of the lytic genes, which is inactivated during lytic induction by the binding of an antirepressor protein called Ant[6]. The regulation of this antirepressor has been previously reported to be under control of the LexA protein (ref Lemire ?). Furthermore, due to the extensive mosaicism shown by this phage family, the same proteins are often used for the regulatory systems of different prophages present in the same *Salmonella* strain, giving place for crosstalk during excision (Lemire).

In this study, the excision rates of Gifsy-1 and Gifsy-2 were assessed by qPCR using the *attB* sites regenerated upon phage excision. Our results showed that in *S. enterica ST4/74* Δ*hns* strain the excision rate for Gifsy-1 increased 8-fold compared to the WT whereas no significant excision change was observed for Gifsy-2. Providing a wild type copy of *hns* carried by a plasmid restored the excision level of Gifsy-1 back to normal. A bioinformatic analysis showed an extensive divergence of the regulatory regions between the Gifsy prophages, which would explain the absence of crosstalk in that strain. Moreover, RT-qPCR assays in the *hns* mutant showed the upregulation of the antirepressor gene *ant*. Its role during lytic reactivation was confirmed by *attB* amplification by qPCR, since a Δ*ant* strain exhibited a decreased excision rate. This phenotype could be complemented by an episomal copy of the wild type gene, showing a sharper increase of the excision rate when the protein was overexpressed.

Overall, this study contributes to shed light on the mechanism involved in the regulation of HGT and the coordination of excision of prophage elements in this *S. enterica* strain.

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Isolation and characterization of the bacteriophages infecting Xanthomonas arboricola pv. juglandis

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Bacterial blight of walnut (*X. arboricola* pv. *juglandis*, Xaj) is one of the most destructive diseases that annually reduces the fruit production in most walnut growing regions in the world. The disease control is challenging, since abundant use of copper-based compounds in walnut orchards resulted in the emergence of highly copper resistant isolates and, therefore, reduced the efficacy of copper-based sprays. Existence of genetic diversity among Xaj strains from different geographical areas in the world is well known and recorded that the geographic location can influence the genomic rearrangement and the heterogeneity of Xaj populations. Some studies also reported that diversity could be linked with the virulence of the pathogen. The aim of this study was to isolate and characterize novel lytic bacteriophages specific to Xaj, with the main idea to evaluate their interaction with pathogenic Xaj strains isolated from different locations in Italy, Serbia and Turkey with diverse virulence. Bacteriophages were collected from different substrates. To increase the potential of Xaj-specific phage isolation, substrate samples were subjected to incubation with target bacteria in 50 ml Nutrient Broth amended with 2.5 g CaCO3. The phage isolates were characterized by host-range, plaque morphology, thermal inactivation and sensitivity to pH, UV light and chloroform. Phage life cycle was studied by a one-step growth procedure. A host specificity assay was performed using 15 bacterial isolates of Xaj collected from symptomatic walnut trees in different years and countries, and possessing a different virulence degree. Parameters, such as country of isolation, type of substrate and different virulence of strains, were evaluated. Extended characterisation of bacteriophages represents, therefore, the basis for the implementation of innovative methods to control the bacterial blight of walnut in commercial groves.

An early expressed Pseudomonas phage protein increases host susceptibility to lysis and antibiotics.

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Bacteriophages (phages) have designed original strategies to alter bacterial physiology and achieve successful viral infection. Molecular mechanisms include protein-protein interactions between early expressed phage proteins and key bacterial targets. Previously, we identified early expressed genes of the virulent myoviridae PAK_P3 by performing transcriptomic analysis during its infection of the opportunistic pathogen *Pseudomonas aeruginosa* [1]. Here we focused on Gp92, a 78 amino acids protein with no homologs outside of few *P. aeruginosa* phages belonging to the same genus.

We discovered that ectopic expression of *gp92* in *P. aeruginosa* alters cells morphology, from rod-shape to cocci-like, without affecting growth rate nor cell viability. Molecular studies revealed that Gp92 possesses an unusual membrane anchoring amino-terminal sequence that is required for this phenotype. To assess the global impact of *gp92* expression on *P. aeruginosa* physiology, we performed a mass spectrometry analysis, which revealed large modifications of the cell proteome. These include the overexpression of RpoH, a heat-shock stress response sigma factor regulating chaperones, which may be required during capside assembly [2].

Next, using a bacterial two-hybrid assay we found that Gp92 interacts with the membrane stress response regulated by the anti-sigma/sigma factors MucA/AlgU. Several assays confirmed that expression of *gp92* lowers the AlgU mediated membrane stress response, suggesting that the role of Gp92 is to control this response during the phage infection. In agreement with this hypothesis, we found that lysis kinetics were affected in membranes stress responses mutants. Therefore, we uncovered a novel strategy for the phage to facilitate bacterial lysis by lowering the host membrane stress response. This observation led to test whether *gp92* expressing *P. aeruginosa* cells could be more susceptible to membrane targeting antibiotics. Indeed, we found that in several strains the MIC for Imipenem decreased by 2 to 4 fold.

Overall, studies on early expressed phage proteins can reveal novel bacterial Achilles' heels (AlgU stress response), a mean to exploit them (Gp92) and could inspire the design of new antibacterial drugs or adjuvants to antibiotics.

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Functional impact of relB-metK region and clpP carried by 12/111phiA prophage on GBS pathogenicity

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Keywords : GBS, temperates phages, biofilm

Abstract :

Context: Group B *Streptococcus* (GBS) is the leading pathogen responsible for neonatal infection particularly CC17 strains described as hypervirulent [1]. We have previously showed an increasing carriage of prophages belonging to a particular group, named A, in GBS strains responsible for neonatal infection during the last 2 decades [2,3]. Comparative study of a CC17 lysogenic strain carrying a prophage A (12/111) and an isogenic prophage-free strain (12/111ΔphiA), revealed significantly higher maximal growth and biofilm formation for 12/111 rather than for 12/111ΔphiA. Using WGS, we have demonstrated that prophages A are mostly inserted close to bacterial genes involved in host adaptation and colonization, i.e., *adaA* and the *murB-potABCD* operon [2,3].

Our hypothesis: 12/111phiA prophagic genes may have an impact on adaptation and pathogenicity of GBS.

Strategy: First, a bioinformatic analysis was performed to identify into the genome of the prophage 12/111phiA prophagic genes potentially involved with fitness of lysogenic strain. Second, two isogenic deleted mutants of potentially interesting regions were constructed by double homologous recombination. Third, biofilm formation was studied for the WT 12/111, the prophage-free strain 12/111ΔphiA and the deleted mutants.

Results: We identified into the genome of the prophage 12/111phiA four ORFs sharing a strong homology with bacterial genes involved in cell persistence and biofilm formation: *relB*, *yafQ*, *clpP* and *metK*. The study of biofilm formation for 12/111, 12/111ΔphiA, 12/111Δ*relB-metK* and 12/111Δ*clpP* revealed a significantly higher Biofilm Formation Index for the lysogenic strain 12/111 compared with prophage-free strain (12/111 vs 12/111ΔphiA, $p \leq 0.001$) and the isogenic deleted mutants (12/111 vs 12/111Δ*relB-metK*, $p \leq 0.01$; 12/111 vs 12/111Δ*clpP*, $p \leq 0.01$)

Conclusion and perspectives. Our data suggest a functional impact of *relB-metK* region and *clpP* carried by 12/111phiA prophage on biofilm formation of GBS CC17 strains. Further investigations are currently performed to elucidate the molecular mechanisms associated with the observed phenotypes.

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Detection of viral particles in bacterial cultures of Xanthomonas campestris pv. campestris

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Bacterium *Xanthomonas campestris*pv. *campestris*(Xcc) (Pammel) Dowson is a causal agent of black rot disease, the most destructive disease of cruciferous plants [1,2]. Since this pathogen is developing resistance against protective chemical compounds, the study of biocontrol agents like bacteriophages have become more attractive and better described [3]. Several studies have been recently focused on the use of viral signature genes to investigate the diversity of viral communities. In this study, we tested available known primer sets for signature genes as g20 Portal protein [4,5], g23 Major capsid protein [6], polA DNA polymerase [7], tyrosine recombinases [8], for detection of different viral families. These primer sets were tested on six natural Xcc isolates collected in Czech Republic. The isolates were divided into two groups – the positive control was represented by bacterial culture and in the second group was induced lysogenic cycle of temperate phages in bacterial culture by Mitomycin C. For each group were employed purification methods to eliminate the bacterial contamination – centrifugation andfiltration. The resulted sequences were compared with GenBank/NCBI and led to designing of the new primers specific to the extant sequences focused on Major Head Protein and Integrase protein. This study brings a new potential marker for recognition of Xcc bacteriophage diversity directly from bacterial cultures.

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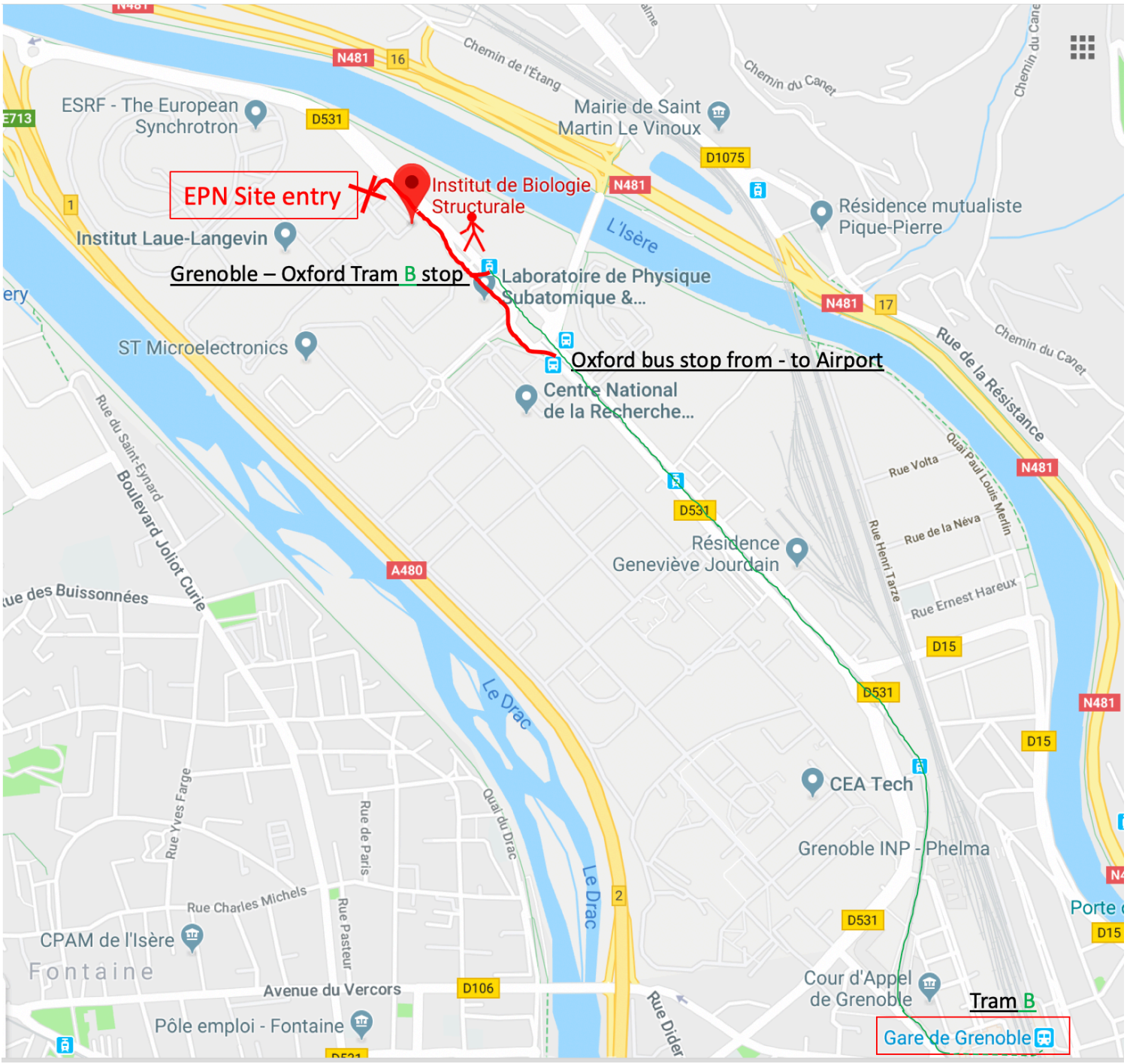

Programme

Tuesday, October 8

8h00	Registration	
8h30	Cécile Breyton	
	Ecology and Evolution	Chair: Damien Piel
8h45	**Evelien Adriaenssens**	
9h20	Frédérique Le Roux	
9h40	Eugen Pfeifer	
10h	Cécile Philippe	
10h20	Coffee Break	
10h50	Marie-Agnès Petit	Chair: Jack Dorling
11h10	Clara Torres-Barceló	
11h30	Hélène Chabas	
11h50	Flash posters, even numbers	
12h10	Lunch	
13h10	Poster session	
	Phage therapy and Biotechnology	Chair: Fernando Clavijo
14h30	**Claire Geslin**	
15h05	David R. Olivenza	
15h25	Ildikó K. Nagy	
15h45	Alexandre Bleibtreu	
16h05	Raphaëlle Delattre	
16h25	Coffee Break	
	Structure and Assembly	Chair: Müge Senarisoy
16h55	**Stefanie Barbirz**	
17h30	Romain Linares	
17h40	Séraphine Degroux	
18h00	Flash posters, odd numbers	
18h20	Poster session	
19h45	Wine & Cheese	

Wednesday, October 9

	Phage-Host Interaction	
8h30	**Calin Guet**	Chair: Maud Billaud
9h05	Marie Vasse	
9h25	Amel Chaïb	
9h45	Pauline Misson	
10h05	Coffee Break	
10h35	Mehdi El Sadek Fadel	
10h55	Luis Ramirez	Chair: Adélaïde Renard
11h15	Naoual Derdouri	
11h35	Audrey Labarde	
11h55	Quentin Lamy-Besnier	
12h15	Kovacs Tamas	
12h30	Lunch	
13h30	General Assembly	
14h30	End	