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Program

Monday November 20th, 2017

09:00 - 09:45	*Registration and Welcome*
09:45 - 10:00	**Introduction and news from the Phage.fr Network**
10:00 - 11:20	**Host-Phage Molecular Interaction** *Chair: Paulo Tavares*
10:00 - 10:40	› Exploiting CRISPR-Cas systems to study phage biology - *Sylvain Moineau, Department of Biochemistry, Microbiology and Bioinformatics, Université Laval, Canada*
10:40 - 11:00	› Phage sensitivity and CRISPR profiles of Escherichia coli strains isolated from the gut of COPSAC2010 cohort children - *Moira Dion, Groupe de recherche en écologie buccale, Département de biochimie, de microbiologie et de bio-informatique, Faculté des Sciences et de Génie, Université Laval, Canada*
11:00 - 11:20	› Bringing to light the dialogue between bacteria and prophages: AppY, a phage-encoded protein central to the bacterial regulatory network? - *Aurélia Battesti, Laboratoire de Chimie Bactérienne, Institut de Microbiologie de la Méditerranée, Marseille*
11:20 - 11:50	*Coffee break*
11:50 - 13:10	**Host-Phage Molecular Interactions** *Chair: Marie-Agnes Petit*
11:50 - 12:10	› Molecular mechanisms of virulent bacteriophages infecting Pseudomonas aeruginosa - *Laurent Debarbieux, Group Interactions Bacteriophages Bacteria in Animals, BMGE Unit, Microbiology Department, Institut Pasteur de Paris*
12:10 - 12:30	› Bacteriophage T5 tail tube structure suggests a trigger mechanism for Siphoviridae DNA ejection - *Cécile Breyton, Institut de Biologie Structurale, Grenoble*
12:30 - 12:50	› Cellular impact of A1, a nuclease of bacteriophage T5 essential for infection - *Ombeline Rossier, Institute for Integrative Biology of the Cell, Gif-sur-Yvette*
12:50 - 13:10	› Characterization of bacteriophage T5 pre-early genes: elucidating the minimal set of genes for host takeover. - *Luis Ramirez, Institute for Integrative Biology of the Cell, Gif-sur-Yvette*
13:20 - 14:30	*Lunch*

14:30 - 16:00	**Ecology and Evolution** *Chair: Mireille Ansaldi*
14:10 - 14:50	› Ubiquitous phage-inducible chromosomal islands in the bacterial universe - *José Penadés, Institute of Infection, Immunity & Inflammation - Biomedical Research Center, Glasgow, UK*
15:10 - 15:20	› Coliphages are prevalent but mostly uninfectious in the infant gut - *Aurelie Mathieu, INRA*
15:20 - 15:40	› Replication of virulent bacteriophages in the gastrointestinal tract: what are we missing? - *Marta Mansos Lourenco, Group Interactions Bacteriophages Bacteria in Animals, BMGE Unit, Microbiology Department, Institut Pasteur Paris, Université Pierre et Marie Curie*
15:40 - 16:00	› Modelling eco-evolutionary interactions between bacteria and bacteriophages - *Jorge Sousa, Microbial Evolutionary Genomics, Institut Pasteur, CNRS, UMR3525*
16:00 - 17:00	*Posters & Coffee break*
17:00 - 17:40	**Ecology and Evolution** *Chair: Nicolas Ginet*
17:00 - 17:20	› What do LE3 and LE4 bacteriophages tell us about Leptospira? - *Olivier Schiettekatte, Unité Biologie des Spirochètes, Institut Pasteur*
17:20 - 17:40	› New insight into the contribution of prophages to the evolution and the pathogenicity of Streptococcus agalactiae - *Nathalie van der Mee-Marquet, UMR 1282, Faculté de Médecine, Tours*
17:40 - 18:00	**"Phages.fr" Network**
17:40 - 18:00	› "Phages.fr" : un site web interactif - *Rémy Froissart, UMR 5290 MIVEGEC*
18:45 - 23:00	**Towards " Domaine de St Paul" by Bus for Dinner - Return at the CNRS campus in Gif-sur-Yvette around 23:00**

Tuesday November 21st, 2017

09:00 - 09:30 *Posters & Coffee break*

09:30 - 10:50 **Host-Phage Molecular Interactions** *Chair: Eduardo Rocha*

09:30 - 10:10 › Farming phages - *Abram Aertsen, Department of Microbial and Molecular Systems, KU Leuven, Belgium*

10:10 - 10:30 › PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data - *Marc Monot, Laboratoire Pathogenèse des Bactéries Anaérobies, Département de microbiologie et infectiologie, Université de Sherbrooke, Canada, Université Paris Diderot, Sorbonne Paris Cité*

10:30 - 10:50 › Molecular mechanisms of viral DNA packaging initiation: recognition and cleavage of the pac site by bacteriophage SPP1 terminase - *Leonor Oliveira, Institut de Biologie Intégrative de la Cellule, Gif-sur-Yvette*

10:50 - 11:50 *Posters & Coffee break*

11:50 - 13:10 **Applications : Therapy and Biotechnology** *Chair: Catherine Schouler*

11:50 - 12:10 › Phage therapy: compassionate use in France in 2017 - *Cindy Fevre, Pherecydes Pharma*

12:10 - 12:30 › Screening for virulent phages against vancomycin-resistant Enterococcus faecium - *Astrid Bouteau, Institut Micalis, INRA*

12:30 - 12:50 › Using genomic approach to assess phage therapy against the plant pest Xylella fastidiosa - *Fernando Clavijo, Laboratoire de Chimie Bactérienne, Institut de Microbiologie de la Méditerranée, CNRS, Aix-Marseille Université, France*

12:50 - 13:10 › Engineering phages and their hosts for the directed evolution of biomolecules - *Alfonso Jaramillo, University of Warwick, UK*

13:20 - 14:30 *Lunch*

14:30 - 15:10 **Applications : Therapy and Biotechnology** *Chair: Ombeline Rossier*

14:30 - 14:50 › Value and limitation of the reductionist principle for microbial biotechnology illustrated with a phage therapy trial - *Harald Brüssow, Division of Animal and Human Health Engineering*, KU Leuven, *Belgium*

14:50 - 15:10 › Pseudomonas aeruginosa phages to disinfect plumbing materials and surfaces in contact with drinking and thermal water : feasibility study - *Nathalie Garrec, Centre Scientifique et Technique du Bâtiment, Nantes*

15:30 - 17:00 **General Assembly of the Phages.fr Network**

Oral presentations

Exploiting CRISPR-Cas systems to study phage biology

Sylvain Moineau [1]
[1] : Department of Biochemistry, Microbiology and Bioinformatics - Faculty of Sciences and Engineering Université Laval, Quebec city - Canada

The year 2017 marked the 100th anniversary of the publication by Félix d'Hérelle, who introduced for the first time the word “bacteriophage” (1). With it, the field of phage biology was born. Viruses are now recognized at the most abundant biological entities on the planet and display a remarkable genetic diversity. Not surprisingly, bacteria have a plethora of diverse defense mechanisms to combat their phages. Four decades after the discovery of one such defense mechanism, restriction-modification systems, another bacterial anti-phage system that cleaves foreign DNA was identified—one that acts as an adaptive immune system. Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated *cas* genes protect microbial cells against infection by foreign nucleic acids, including phage genomes and plasmids. Bacterial CRISPR-Cas type II systems function by first incorporating short DNA ‘spacers', derived from invading phage genomes or plasmid sequences, into a CRISPR array located in their genome. This step is known as adaptation or vaccination. The CRISPR array is then transcribed and matured into short RNAs (the maturation step), which, by recruiting a Cas endonuclease, act as surveillance complexes that recognize and cleave invading matching sequences (the interference step). For some systems, the cleavage requires a short motif, called the PAM, close to the sequence targeted by the spacer. Exploiting this system has also resulted in the development of the much-publicized CRISPR-Cas9 technology for precise genome manipulation of various organisms. This seminar will recall the roles played by phages in the discovery and understanding of CRISPR-Cas systems. I will also highlight the recent emergence of virulent phages capable of inactivating the CRISPR-Cas system through anti-CRISPR proteins (2) as well as the use of CRISPR-Cas9 technology for viral genome editing in order to better understand phage-host interactions (3). Finally, I will briefly present the use of CRISPR-Cas systems and phages as teaching tools (4).

1- Félix d'Hérelle, F. 1917. Sur un microbe invisible antagoniste des bacilles dysentériques. *Comptes Rendus de l'Académie des Sciences* 165:373-375.

2- Hynes, A.P., G.M Rousseau, M.-L. Lemay, P. Horvath, D. Romero, C. Fremaux, and S. Moineau. An anti-CRISPR from a virulent streptococcal phage inhibits SpCas9. Nature Microbiology. DOI: 10.1038/s41564-017-0004-7.

3- Lemay, M.-L., D. Tremblay, and S. Moineau. 2017. Genome engineering of virulent lactococcal phages using CRISPR-Cas9. ACS Synthetic Biology. 21:1351-1358.

4- Trudel, L., M. Frenette, and S. Moineau. 2017. CRISPR-Cas in the laboratory classroom. Nature Microbiology. 2:17018

Phage sensitivity and CRISPR profiles of Escherichia coli strains isolated from the gut of COPSAC2010 cohort children

Moira Dion [1], Denise Tremblay [1], Shiraz Shah [2], Jakob Stokholm [2], Ling Deng [3], Josue Leonardo Castro Mejia [3], Dennis Sandris Nielsen [3], Hans Bisgaard [2], Sylvain Moineau [1]

[1] : Groupe de recherche en écologie buccale, Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval
[2] : Copenhagen Prospective Studies on Asthma in Childhood, Copenhagen University
[3] : Department of Food Science, Copenhagen University

In light of its crucial role in human health, the gut microbiota has been extensively studied in the last decade. A very diverse and abundant population of phages also colonizes the gut and is referred to as the gut virome. However, the literature on the gut virome is largely centered around viruses fortuitously sampled through metagenome analyses mostly targeted at the bacteria. Techniques for extracting and sequencing viral particles from such complex environments are also still embryonic. Thus, it remains challenging to explain the roles of the phages, the identity of their hosts and their interactions in the gut. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) loci, together with their *cas* genes (CRISPR associated genes) are found in nearly half of the bacterial genomes sequenced so far. They provide adaptive immunity against invading genetic elements by incorporating short DNA sequences in the locus, called spacers, in an orderly fashion. These spacers often originate from the phage genome and allow the bacterium to block a subsequent infection. The integration of new spacers is primarily localized on one end of the locus, similar to adding a new page to a book. This characteristic, combined with the rapidly-evolving structure of the locus, can serve as a molecular archive of past phage-bacteria interactions.

We sequenced the two most active CRISPR loci of 1871 *Escherichia coli* isolates originating from 700 children and their mothers enrolled into the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) cohort, sampled during the third trimester (vaginal samples) and 1 week, 1 month and 1 year after birth (feces samples). By looking at the spacers content of the loci, we attributed a CRISPR signature for each isolate. We observed a clear bacterial transfer from the mother to the child and occasional spacers acquisition and deletion over time. Total gut virome has also been extracted and metavirome sequenced from a subset of the cohort. 16 of all 1763 distinct spacers matched a sequence retrieved from the virome. At this time, we have challenged 1769 *E. coli* isolates with 16 well-characterized coliphages to determine their phage sensitivity profile. Some bacteria were immune to all phages, while others were sensitive to more than six distantly related phages. Interestingly, we found no correlation between the CRISPR signature and the phage sensitivity profile, emphasizing the idea that other defense mechanisms are at play for *E. coli* to defend themselves against phages in the gut ecosystem.

Bringing to light the dialogue between bacteria and prophages: AppY, a phage-encoded protein central to the bacterial regulatory network?

Aurélia Battesti [1] ,Nicolas Ginet [1] , Mireille Ansaldi [1].

[1] : Laboratoire de chimie bactérienne, UMR7283, Institut de Microbiologie de la Méditerranée (LCB) - Marseille, France

Bacterial genomes are extremely diverse. Part of this diversity is due to temperate bacterial viruses that can integrate into and maintain themselves in the host genome; once integrated, they become prophages. In bacteria, although most of the prophage genes are silent, some of them are expressed and give new properties to bacteria such as stress adaptation and virulence factors.

Recently, it has been shown in a genetic screen that the overproduction of AppY, a poorly studied transcriptional regulator encoded by the DLP12 prophage in *Escherichia coli* K12, leads to RpoS increase in the cell (Bougdour *et al*., 2008). RpoS is the major sigma factor during stationary phase and under many stress conditions in γ-proteobacteria. This factor controls the expression of more than 500 genes in *E. coli* and is itself highly regulated, in particular *via* changes in protein stability. Indeed, once produced, RpoS is actively degraded by the ClpXP protease. To be recognized as a protease substrate, RpoS interacts with an adaptor protein called RssB, which brings RpoS to the degradation machinery. My recent results suggest that AppY stabilize RpoS through a direct interaction with the RssB adaptor protein, an interaction that I intent to characterize precisely.

Moreover, it has been suggested that AppY could regulate around 30 genes in the cell (Atlung *et al*., 1989). In order to identify these potential AppY targets, we have performed RNA-seq experiments. Our results suggest a broader role for AppY in the bacterial physiology than previously anticipated. Overall, the objective of my research is to bring to light the regulatory pathways existing between genes from bacterial and phage origins and determine how these regulations contribute to bacterial physiology and adaptation to different stress conditions.

- Bougdour et al., 2008. *Mol. Microbiol*. 68(2):298-313.

- Atlung *et al*., 1989. *J. Bacteriol*. 171(3) : 1683-1691

Molecular mechanisms of virulent bacteriophages infecting Pseudomonas aeruginosa

Laurent Debarbieux [1], Anne Chevallereau, Mathieu De Jode, Elsa Brambilla, Gouzel Karimova

[1] : Group Interactions Bacteriophages Bacteria in Animals, BMGE Unit, Microbiology Department, Institut Pasteur de Paris, France

Pseudomonas aeruginosa bacteriophages PAK_P3 and PAK_P4 belong to two distantly related genera of *Caudovirales* viruses. Outside the cluster of genes coding for structural proteins that are almost identical, the rest of the genome is highly divergent. Nevertheless, few open reading frames display a strong conservation within bacteriophages belonging to these two genera suggesting they may code for conserved functions.

In this work we examined one of these conserved ORFs which displays a phenotype of growth defect when expressed ectopically in the bacterial host. Interestingly, this phenotype was also observed when expressed in two other *P. aeruginosa* hosts as well as in *Escherichia coli*. Using a two-hybrid system in *E. coli* we identified the anti-σE factor RseA as a putative interacting partner of the product of this ORF. The *P. aeruginosa* homologous protein of RseA is named AlgU, while the σE factor is named MucA. Both AlgU and MucA are well studied proteins in *P. aeruginosa* as being associated to the general stress response that regulates in particular the biosynthesis of alginate, which is linked to the mucoid phenotype often found in clinical isolates from cystic fibrosis patients. We experimentally verified that the product of this ORF is interacting directly with AlgU and are proposing a mechanism used by the bacteriophage to modulate the stress response in order to achieve its infectious cycle.

Bacteriophage T5 tail tube structure suggests a trigger mechanism for Siphoviridae DNA ejection

Cécile Breyton [1]
[1] : Institut de Biologie Structurale (IBS - UMR 5075) - Grenoble, France

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 proteins (RBP). Little is known on the mechanisms that trigger DNA ejection after binding of *Siphoviridae* to their host. Here, we report the overall structure of siphophage T5 tail tube by determining the crystal structure of T5 TTP pb6 at 2.2 Å resolution and the structure of T5 tail tube by cryo electron microscopy (EM) at 6Å resolution. Fitting the crystal structure of the TTP into the EM map made it possible to propose a pseudo-atomic model of the tail tube. We also observe that the structure of the tail tube remains unchanged after interaction of T5 tails with the host receptor, showing that host binding information is not propagated to the capsid by the tail tube. We rather suggest that the TMP, folded in a metastable state, would transmit the signal. RBP-host receptor interactions and subsequent tail tip rearrangements would destabilise the TMP, leading to its ejection from the tube and ultimately to capsid opening and DNA release. Although pb6 is unusually formed of three domains, structure analysis reveals homology of pb6 with all classical TTPs and related tube proteins of bacterial puncturing devices (type VI secretion system and R-pyocin) extending thus our findings to most of biological tailed-like structures.

Cellular impact of A1, a nuclease of bacteriophage T5 essential for infection

Ombeline Rossier [1], Léo Zangelmi [1], Madalena Renouard [1], Pascale Boulanger [1]
[1] : Institute for Integrative Biology of the Cell (I2BC) - UMR9198, CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, - Gif-sur-Yvette, France

Phage T5 infects *Escherichia coli* and injects its genome in an original two-step mechanism: in the First Step Transfer (FST), only 8% of the phage DNA enters the host cell. The transfer pauses then for several minutes before DNA entry resumes to completion (Second Step Transfer, SST). The FST is accompanied by a very rapid and massive destruction of bacterial DNA (50 % decrease in labeled DNA within 4 min of infection). The identity of the phage T5 nuclease has remained elusive for sixty years, as none of the phage proteins encoded on the FST-DNA resemble known nucleases. However, *A1*, a gene carried by FST-DNA, appears to control host DNA degradation as well as the SST. The C-terminal half of A1 carries several motifs that are conserved in a large family of metallophosphatases including the DNA repair and recombination nucleases Mre11/SbcD/gp46. Purified A1 exhibits manganese-dependent DNase activity on linear or plasmid DNA *in vitro*. In this study we investigated the role of the 62-kDa protein A1 in DNA degradation in the bacterial cell.

Upon ectopic expression of *A1* (cloned under the control of an arabinose-inducible promoter), there was a dramatic decrease in genomic DNA recovered from bacterial cultures. Using fluorescence microcopy of *E. coli* cells, we observed a rapid decrease in bacterial DNA staining with DAPI within 15 min of induction. Moreover, we frequently saw the formation of fluorescence foci, suggesting a major reorganization of the bacterial nucleoid. Mutations in putative catalytic amino-acid residues abolished nuclease activity in vitro as well as in vivo. Taken together, our results indicate that A1 is the long elusive early-encoded DNase of phage T5. Interestingly, T5 phage DNA is not modified and is sensitive to A1 *in vitro*. How the DNase activity of A1 is regulated to control the SST without digesting the T5 genome remains to be elucidated.

Characterization of bacteriophage T5 pre-early genes: elucidating the minimal set of genes for host takeover.

Luis Ramirez [1], Ombeline Rossier [1], Pascale Boulanger [1]
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T5 is a lytic bacteriophage that infects *Escherichia coli*. T5 genome delivery is carried out in two steps:(1) first, only 8% of the whole genome enter the host cell. The corresponding phage pre-early genes are quickly expressed: they inactivate host defense systems, and trigger massive host genome degradation. Then, during the second step, the remaining genome, including mid and late genes, is transferred into the host, thus resuming the phage replication cycle. Because of this unique mechanism of DNA delivery, T5 is an attractive model to study the role of pre-early genes in the host takeover process.

The First Step Transfer (FST) region, injected during the first step, includes sixteen genes. Thus far, only two of them, A1 and A2, are known to be essential for infection. A1 has DNase activity in vitro and in vivo, while A2 seems to act as a transcriptional regulator. However, it is still not clear whether the rest of the FST genes are dispensable and what their function is.

In addition to A1 and A2, genome analysis identified six FST genes, which are conserved in almost all T5-like phages: 02, 05, 07, 10, 13, 14. To assess the role of 02, 05 and 07, we are currently using two strategies: (i) studying the effects of gene deletion in the T5 genome on the infection process and (ii) testing the impact of phage gene overexpression over host fitness.

To accomplish the first aim, we optimized the CRISPR/Cas9 system (2) to select T5 mutants engineered by homologous recombination and we improved the yields up to 1 mutant per 10 lysis plaques. T5 mutants deleted in genes 02 or 05 were obtained, indicating that both genes are not essential for infection under laboratory conditions. We are currently studying their impact on T5 infection kinetics.

In our second parallel approach, we found that overexpression of gene 05, but not genes 02 or 07, inhibits growth of *E. coli*. These results suggest that the putative transmembrane protein 05, while not essential to T5 multiplication, might be toxic to the host during infection. Future work will aim at understanding the function of the minimal set of T5 pre-early genes in the host takeover.

1. Davison, J. Pre-early functions of bacteriophage T5 and its relatives. *Bacteriophage* **5,** e1086500 (2015).

2. Lemay, M.-L., Tremblay, D. M. & Moineau, S. Genome Engineering of Virulent Lactococcal Phages Using CRISPR-Cas9. *ACS Synth. Biol.* (2017). doi:10.1021/acssynbio.6b00388

Ubiquitous phage-inducible chromosomal islands in the bacterial universe

José Penadés [1]
[1] : Institute of Infection, Immunity & Inflammation - Biomedical Research Center - University of Glasgow, UK

Ourselves and others have discovered and extensively characterised a family of pathogenicity islands in Gram-positive cocci, the phage-inducible chromosomal islands (PICIs), which contribute substantively to horizontal gene transfer, host adaptation and virulence. We now report that similar elements also occur widely in Gram-negative bacteria. As with the PICIs from the Gram-positive cocci, their uniqueness is defined by a constellation of features: unique and specific attachment sites, exclusive PICI genes, a phage-dependent mechanism of induction, conserved replication origin organization, convergent mechanisms of phage interference, and specific packaging of PICI DNA into phage-like infectious particles, resulting in very high transfer frequencies. We suggest that the PICIs represent two or more distinct lineages, have spread widely throughout the bacterial world, and have diverged much more slowly than their host organisms or their prophage cousins. Overall, these findings represent the discovery of a universal class of mobile genetic elements, which have had a broad impact on lateral gene transfer in the bacterial world.

Coliphages are prevalent but mostly uninfectious in the infant gut

Aurelie Mathieu [1], Denise Tremblay [2], Ling Deng [3], Martin Mortensen [4], Jakob Stokholm [4], Dennis Sandris Nielsen [3], Sylvain Moineau [2], Hans Bisgaard [5], Marie-Agnès Petit [1]
[1] : INRA- Institut National de la Recherche Agronomique
[2] : Université Laval
[3] : University of Copenhagen, food.
[4] : University of Copenhagen, microbiology
[5] : COPSAC

A link between one-year old infant gut microbiota and asthma predisposition starts to emerge from several studies (1, 2). Since bacteriophages constitute an important component of the gut microbiome, it is of interest to investigate if they could influence early life microbiota colonization and maturation and whether they play a role in the risk of asthma development. The international joint initiative ‘Earlyvir', aims at studying the infant viromes.

Escherichia coli is one of the first bacterial species to colonize the infant's gut. This population is highly abundant (~15% of OTU), quite stable during the first month of life, and decreases thereafter (5% at 1 year). This dynamic evolution and the possibility to collect strains from feces, make this specie a good model to start studying the phage-bacteria interactions in the gut.

We used the *E.coli* collection of the COPSAC2010 cohort (3), in which fecal samples of 700 children were collected and a total of 1769 *E.coli* strains were isolated at 1 week (348 isolates), 1 month (467 isolates) and 1 year (954 isolates). We quantified the number of strains containing active temperate phages as well as their level of phage particle production. We find that 62.2% of these *E.coli* strains contain active temperate phages, ie forming plaques on laboratory strains *E.coli* C and MG1655. This percentage evolves over time suggesting two different *E.coli* population dynamics. We also show that during this period, *E.coli* strains containing active prophages are not selected depending on their level of phage production but more probably according to their host spectrum. We next analyzed the capacity of 90 of these phages, to infect 90 COPSAC *E.coli* strains. 79% of *E.coli* strains are resistant to all temperate phages, showing that *E.coli* isolates from the gut possess efficient barriers to protect themselves from temperate phages produced by their neighbours. The genome of twenty temperate phages from *E.coli* strains isolated at 1-year were also sequenced. Sequence analysis showed two main clusters (lambda-like and P2-like) and two minor clusters (Mu-like, P88-like).

Finally, we studied 153 viromes extracted from fecal samples at 1 year. At least 20% of these viromes contain coliphages and our first results suggest that natural *E.coli* strains are also resistant to these phages.

Therefore, we observe a contrast between high prevalence of coliphages in the infant gut and their low infectivity against residing population. These phages might be useful however to fight against *E.coli* invaders.

Replication of virulent bacteriophages in the gastrointestinal tract: what are we missing?

Marta Mansos Lourenco [1,2], Luisa De Sordi [1], Laurent Debarbieux [1]
[1] : Group Interactions Bacteriophages Bacteria in Animals, BMGE Unit, Microbiology Department, Institut Pasteur de Paris
[2] : Université Pierre et Marie Curie, ED-Complexité du Vivant

The rapid proliferation of multi-drug-resistant bacteria has grown into a critical problem in the last few years. To tackle this problem, our lab investigates the potential of bacteriophages (phages) to treat bacterial infections. Although much is known about phage biology *in vitro*, the physiological, ecological and evolutionary interactions between phages and bacteria in natural environments are still poorly understood.

Up to now, the use of virulent bacteriophages to significantly reduce the levels of targeted bacteria in the gastrointestinal tract (GIT) has often been disappointing, showing at best a moderate effect in contrast to the high efficiency observed *in vitro*. These observations suggests the existence of some factors that can modulate the activity of phages in the GIT.

In order to uncover these factors, phage replication was first assessed in different specific conditions: *in vitro* during both exponential and stationary phase cultures, and *ex-vivo*, using homogenized different gut sections of mice colonized with the targeted bacteria. We observed that while some phages replicate efficiently regardless of the section of the GIT or the state of the *in vitro* cultures, others display a markedly different efficiency that depends on the environment. We hypothesized that the local gut environment may influence the physiological state of the bacterial host, which in turn can impact the efficiency of the phage infection/replication.To further test this hypothesis, we performed comparative genome-wide RNA-sequencing analysis of the *Escherichia coli strain 55989* growing in the different sections of the GIT as well as *in vitro* cultures, during exponential and stationary growth phases. We have identified more than 50 candidate genes that are significantly differentially expressed and might be involved in phage efficiency, supporting a possible connection between the physiological state of the bacteria and the outcome of the phage infection.

The characterization of interactions between phages and bacteria in their natural environment is key to increase knowledge in phage biology and could be useful to design better strategies that can improve the overall efficiency of Phage Therapy.

Modelling eco-evolutionary interactions between bacteria and bacteriophages

Jorge Sousa [1,2], Marie Touchon [1,2], Eduardo Rocha [1,2]
[1] : Microbial Evolutionary Genomics, Institut Pasteur de Paris
[2] : CNRS, UMR3525

Bacteriophages are one of the most ubiquitous entities in nature and play a crucial role in the dynamics of microbial communities. They can act both as bacterial predators, directly shaping which bacterial species are present in the environment, and also as drivers of horizontal gene transfer, promoting the dissemination of adaptive traits through transduction. An extensive amount of work has been done, both experimentally and theoretically, to understand the conditions in which certain bacterial species are favoured or disfavoured by the presence of bacteriophages. Nevertheless, it is difficult to study the role of the different mechanisms involved in the interactions between bacteria and bacteriophages, particularly in ecologically complex scenarios, where spatial structure can greatly influence the dynamics of the communities.

We developed an ecological and evolutionary individual-based model to better understand the impact of bacteriophages in microbial communities. Each bacteria and bacteriophage are explicitly modelled, and we are able to track the fate and individual behaviours of each cell, their adaptation at the genomic and phenotypic level and the stochasticity inherent to the genetic mechanisms underlying their interactions. Our model recovers the typical observed dynamics of bacteria-bacteriophage interactions, whilst simultaneously predicting the outcome of more ecologically complex scenarios of adaptation. In particular, we simulate the dynamics of phage predation in spatially distributed communities, the different roles of lysogeny in these communities, and the effect of transduction in bacterial adaptation.

The mechanistic-based theoretical approach we propose here can be inspired by results from experimental approaches and comparative genomics, but it can also guide further research. It is an important step to integrate the different scales involved in the study of bacteria-phage interactions, from the individual molecular processes to the collective, community level dynamics. Such integrative approaches will be fundamental to disentangle these interactions and their consequences for microbial communities and public health.

What do LE3 and LE4 bacteriophages tell us about Leptospira?

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Leptospirosis is a zoonotic disease caused by infection with pathogenic species of the genus *Leptospira*—Gram-negative bacteria belonging to the *Spirochaetes* phylum. To date 22 *Leptospira* species are known in this genus; including saprophytes and pathogens which have the ability to persist in freshwater. We have isolated three bacteriophages of *Leptospira* (leptophages) called LE1, LE3 and LE4. All of them are specific to the saprophyte *Leptospira biflexa* and they belong to the *Myoviridae* family. Interestingly, prophage-like regions have been found in infectious strains but not in saprophytes. The genome of the lysogenic phage LE1 was previously sequenced (74kb) and its origin of replication was used to design a shuttle vector. The lytic phages LE3 and LE4 are characterized in this study in order to further evaluate the diversity of phages and prophages in the leptospires.

LE3 and LE4 have a similar morphology and their genomes are closely related. The 48kb genome of LE4 contains 81 open reading frames (ORFs). Putative functions could be assigned to only 12 gene products (15% of gene content) based on sequence homology and motif searches. Additionally, mass spectrometry analyses were performed on culture supernatants and we were able to consistently identify 11 gene products of phage origin. In order to identify a bacterial LE4 receptor, we selected a phage-resistant *L. biflexa* mutant strain called RLE4. Whole-genome sequencing of RLE4 allowed the identification of a single nucleotide polymorphism in a gene of the O-antigen locus that introduced a premature stop codon. This result was confirmed by targeted mutagenesis of the aforementioned gene in the WT strain, and suggests that the lipopolysaccharide is a receptor for the phage.

Finally, using comparative genomics, LE3 and LE4 exhibited homologies with other prophages in the published *Leptospira* genomes, which could, together, form a new bacteriophage/prophage group in *Leptospira*. Moreover, we identified a new LE4-like circular prophage, maintained as a plasmid, in the pathogen *L. mayottensis*.

Sequencing of these two leptophages will advance several aspects of the field including improved detection methods of prophages in *Leptospira* genomes, development of genetic tools, identification of novel genes, prediction of hypothetical proteins, and better understanding of phage contribution to bacterial evolution and virulence.

Prophages et pathogénicité de Streptococcus agalactiae chez l'homme

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CONTEXTE. Streptococcus agalactiae (GBS) est depuis les années1970s le principal pathogène responsable d'infections sévères du nouveau-né. De plus, depuis les années 1990s, il est associé à un nombre croissant d'infections invasives chez l'adulte. Pour investiguer le rôle possible de la lysogénie dans ces 2 évolutions épidémiologiques récentes, nous avons caractérisé le contenu prophagique de 275 isolats humains représentatifs de la diversité de l'espèce.

METHODES. (1) Nous avons séquencé le génome de 14 isolats, localisé et identifié leurs prophages et recherché les gènes prophagiques potentiellement impliqués dans la capacité de GBS à infecter l'homme; (2) nous avons mis au point un outil PCR pour cartographier précisément le contenu prophagique des souches; (3) à l'aide de cet outil, nous avons étudié le contenu prophagique de 275 isolats.

RESULTATS. Le séquençage des 14 isolats a identifié 22 prophages distribués en 6 groupes (A-F) Les séquences prophagiques (i) présentent des similitudes avec les séquences de phages et de prophages de GBS et d'autres streptocoques d'origine animale, et (ii) portent des gènes codant pour des facteurs associés à l'adaptation à l'hôte ou à la virulence. L'étude du contenu prophagique des 275 souches a montré (i) 72.4% des isolats portant au moins un prophage, et (ii) une association significative entre isolats responsables d'infection néonatale et prophages C, et entre isolats responsables d'infection chez l'adulte et prophages A.

CONCLUSIONS. Nos résultats suggèrent un rôle des prophages (peut-être d'origine d'animale) dans l'adaptation de GBS à l'homme, et l'accroissement de sa capacité à induire des infections invasives chez le nouveau-né et chez l'adulte.

"Phages. fr" : un site web interactif

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Suite à la mise en ligne du site web http://phages.fr présentant les acteurs et les activités de notre réseau thématique pluridisciplinaire (RTP), je ferai une présentation synthétique du site et de la manière de l'alimenter. Nous présenterons aussi les liens vers les sites de notre RTP constitués sur les réseaux sociaux (Facebook, Twitter, ResearchGate, Linkedin, etc).

Farming phages

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Phages have been shown to be a driving force in shaping the behavior and evolution of their host. However, subtle but meaningful phage-host interactions that are heterogeneous or transient in nature likely tend to be overlooked with population level approaches. By studying phage infection dynamics with single cell resolution, the different possible fates and whereabouts of individual host cells and phage chromosomes throughout an active infection can be monitored in great detail. In the P22 – *Salmonella*Typhimurium model system, this approach revealed the existence of a phage carrier state causing the emergence of a transiently resistant subpopulation that seems to support phage – host coexistence. These intricate dynamics fuel the notion that phages have evolved to farm host populations.

PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data

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The worrying rise of antibiotic resistance in pathogenic bacteria is leading to a renewed interest in bacteriophages as a treatment option. Novel sequencing technologies enable description of an increasing number of phage genomes, a critical piece of information to understand their life cycle, phage-host interactions, and evolution. In this work, we demonstrate how it is possible to recover more information from sequencing data than just the phage genome. We developed a theoretical and statistical framework to determine DNA termini and phage packaging mechanisms using NGS data. Our method relies on the detection of biases in the number of reads, which are observable at natural DNA termini compared with the rest of the phage genome. We implemented our method with the creation of the software PhageTerm and validated it using a set of phages with well-established packaging mechanisms representative of the termini diversity, i.e. 5'cos (Lambda), 3'cos (HK97), pac (P1), headful without a pac site (T4), DTR (T7) and host fragment (Mu). In addition, we determined the termini of nine Clostridium difficile phages and six phages whose sequences were retrieved from the Sequence Read Archive.

PhageTerm could also be used to detect whether a contigs reconstructed from virome data has a phage origin or not. An issue with contigs from virome data is to sort phage sequences from background (host sequence, assembly artefact...). For this purpose, current available methods (Phaster, MetaPhinder, Virsorter...) are all based on sequence homology. PhageTerm is the only one to our knowledge that is not based on homology as it detects termini using biases in the number of sequencing reads. Thus, we tested PhageTerm on contigs obtained with virome data and it was able to detect termini on severals contigs. PhageTerm found termini on contigs detected by other software but also on contigs that are never defined as phage sequence by other software.

PhageTerm is freely available (https://sourceforge.net/projects/phageterm), as a Galaxy ToolShed and on a Galaxy-based server (https://galaxy.pasteur.fr).

Molecular mechanisms of viral DNA packaging initiation: recognition and cleavage of the pac site by bacteriophage SPP1 terminase

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Specific recognition of the viral genome is an essential step in the assembly of most viruses, including tailed bacteriophages. In numerous tailed phages, such as SPP1, genome packaging is initiated by recognition and cleavage of a specific sequence pac, by the small (TerS) and large (TerL) terminase subunits. Cleavage at pac occurs only once, initiating a packaging series along a substrate concatemer. The packaging cycle is terminated by a non-specific sequence cleavage determined by the amount of DNA inside the capsid, yielding packaged molecules longer than the phage genome (headful packaging mechanism). It was previously shown that the SPP1 pac region has two sequences where TerS binds (pacR and pacL) flanking the segment where TerL cleaves the SPP1 DNA (pacC). However, the pac specific sequences required to achieve this endonucleolytic cut were not established. Their characterization is essential to understand the underlying mechanism. In this study we used a plasmid minimal system encoding SPP1 pac, TerS and TerL that mimics specific pac recognition and its auto-regulated cleavage in Bacillus subtilis. the SPP1 host. We show that the pacR sequence localized within 35 bp downstream of the pac cut can be extensively degenerated, including its c1 and c2 repeats, and that only disruption of a 5 bp polyadenine tract impairs pac cleavage. This result together with deletion analysis of pacL shows that the specific DNA sequences required for targeting the terminase for pac cleavage are considerably shorter than the large region bound by TerS. Furthermore, extensive degeneration of the 6 bp target sequence within pacC where pac cleavage occurs, reveals that TerL maintains, remarkably, its precise position of cleavage. Studies with SPP1-related phages show conservation of the cut position, irrespectively of sequence variation in pacC, in pacR or changes in pacL-pacC distance. Mechanistically our data are compatible with a model in which TerS interactions with part of the pacL sequence and a poly-A tract in pacR are sufficient to orient very accurately the TerL nuclease to a defined *pac*C position. They also demonstrate that the resulting precise cut at *pac*C is independent of the targeted DNA sequence.

Phage therapy: compassionate use in France in 2017

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Phages: an old drug to fight emergent resistant infections. In the last decade this statement has been published many times. Although scientist working on phages are convinced that phage therapy is a very promising alternative to antibiotics, no phage drug is yet approved in western countries.

We propose to present the current regulatory status of therapeutic phages in France, and the recent advances that allowed patients undergoing therapeutic failure to benefit from experimental therapeutic phages in Lyon, at the beginning of 2017.

Two patients with severe bone and joint infections due to resistant pathogens were admitted to the “Hospices Civils de Lyon” (HCL) in France. As conventional drugs were not efficient, and because the patients were at risk to lose their motor skills and mobility, the treating physicians, under the supervision of the French National Agency for Medicines and Health Products Safety (ANSM), decided to administrate therapeutic phages. Based on a preliminary diagnostic, a phagogram, active phages were selected and produced by the French Company Pherecydes Pharma. After a thorough examination of the therapeutic phages characteristics and of the data of quality control tests by experts of the regulatory agency and pharmacists of the HCL, the phages were administered locally.

No side effect related to the treatment were reported, and the infection symptoms vanished. These promising clinical results pave the way to the new phage therapy, including through other administration routes than local administration.

Screening for virulent phages against vancomycin-resistant Enterococcus faecium

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Enterococcus faecium is a Gram-positive facultative anaerobe bacterium that commensally colonizes the human intestinal tract. It is part of the normal gut microbiota and has normally no adverse effects on healthy individuals. However, *E. faecium* can also become an opportunistic pathogen and has emerged as a leading cause of nosocomial infections, particularly in immunocompromised patients. The clinical importance of *E. faecium* is directly related to its antibiotic resistance. The bacterium has been very successful at rapidly acquiring multi resistance to antimicrobial agents commonly used in therapy. The rapid spread of vancomycin-resistant *E. faecium* (VRE) clinical isolates has been of particular concern and associated hospital acquired infections have become a growing problem. The use of bacteriophages could be a therapeutic alternative to combat VRE infections. However, the number of characterized and potential interesting virulent phages still remains scarce in literature, with five published genomes belonging to 3 different clades.

In this study, we performed a phage-screening on 14 *E. faecium* VRE clinical isolates, provided by the National Reference Center of Caen (CNR). Four raw sewage water samples from Ile de France-wastewater treatment plants (provided by the Pasteur Institute) were used as a source of phages. Following culture-enrichment and isolation steps, we were able to isolate 23 phages growing on 6 of the 14 VRE. Spot assays revealed that isolated phages have host ranges of 1-5 strains and encompass a total of 8 VRE. Interestingly, 5 of these 8 belong to the most clinical prevalent sequence types ST 17 and ST 18. Based on distinct plaque morphologies and host ranges, we selected 10 of the 23 phages for analyses on transmission electron microscopy. All belong to the *Caudovirales*-order and members all 3 families *Sipho*-, *Myo*- and *Podoviridae* were observed. A phage affiliated to each one of these families was subsequently chosen for further characterization. Primary results indicate these 3 phages, VRE-s1 (siphophage), VRE-m1 (myophage) and VRE-p1 (podophage), have distinct burst sizes (29, 122 and 58, respectively) and latent periods (15, 45 and 40 min, respectively). Genomic analyses show that while VRE-s1 and VRE-m1 are highly homologous to 2 phages already described for the closely related *Enterococcus faecalis* species, VRE-p1 could be a member of a new genus within *Picovirinae* subfamily.

Using genomic approach to assess phage therapy against the plant pest Xylella fastidiosa

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Xylella fastidiosa is a Gram-negative plant-pathogenic bacterium emerging in Asia and Europe. Long-known in the Americas, this xylem-limited bacterium is associated with several socio-economically important plant diseases and it is transmitted by xylem-feeding insects. Nowadays, *X. fastidiosa* has been introduced in various places of the Mediterranean regions probably through commercial exchanges (Jacques et al. 2016). Listed as a quarantine pest in Europe, three subspecies of *X. fastidiosa* were recently detected in Europe. First, the subspecies *pauca*, the causal agent of Citrus Variegated Chlorosis (CVC) affecting Citrus and Coffee trees in the USA, has been associated with the Leaf Scorch of Olive trees in Italy since 2013. Another strain from this subspecies was also recently detected in Mallorca, Spain. *X. fastidiosa* subsp. *multiplex*, including strains causing diseases in Oleander and Polygala, has been detected in the south of France and in Corsica (2015) and in Spain (2016). And, finally, the subspecies *fastidiosa*, the causal agent of the Pierce's disease on Grapevines in the USA, was detected on oleander in Germany and in Sweet Cherry plants in Spain (2016).

Bacteria frequently acquire new genes and functions through horizontal gene transfer. We are particularly interested by integrated forms of temperate phages, since in many cases prophages contribute to the host fitness and physiology, as well as to bacterial virulence (Fortier et al., 2013). Prophages are also involved in various mechanisms behind resistance to infection by other phages, which is an obvious disadvantage considering phages as biocontrol agents (Bondy-Denomy et al., 2016). Interestingly, despite their relative small sized-genomes, *X. fastidiosa* species host numerous prophages suggesting the latter may contribute to the host physiology (Varani et al. 2013).

Important goals of our project are: (i) to identify prophages genes in *X. fastidiosa* genomes; (ii) to make a link between prophages and host range specificity of virulent phages, and (iii) to determine phage contribution to strain subsets emergence. Using a bioinformatics approach, we analyzed 39 genomes from six subspecies (*pauca*, *multiplex*, *fastidiosa*, *sandyi* and *morus*) representing at best the know diversity of *X. fastidiosa*.

With this genomic approach we hope to bring some light in the understanding of the contribution of integrated-phage genomes in the fitness and pathogenicity of *X. fastidiosa*.

Engineering phages and their hosts for the directed evolution of biomolecules

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In vivo directed evolution techniques allow engineering protein and nucleic acids with targeted functions inside living cells. The efficiency of such techniques is determined by the evolution speed and sampling size inside the organism. Viruses with fast replicative cycles and able to support high mutagenesis rates allow implementing a faster evolution, where the host cell is re-engineered according to the desired selection. Phages are specially suited due to their small size, fast replication and the ease of engineering of their genomes and their hosts. We have developed directed evolution systems based on filamentous (M13) and lytic (T7) phages. We have engineered their genomes and hosts by removing from the phages genes required for their replication to later complement them within the host. The implementation of negative selections allowed the engineering of specificity. We demonstrate the usefulness of our system by engineering the largest known set of orthogonal transcription factors able to activate and/or repress cognate or combinatorial promoters in E. coli. We also show how to evolve riboswitches using cycles of positive and negative selections. Our methodology for directed evolution can be implemented in many phage systems to evolve proteins, nucleic acids and phage tropism determinants.

Value and limitation of the reductionist principle for microbial biotechnology-illustrated with a phage therapy trial

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One of the most fruitful concepts of biology was the reductionist principle. The detailed study of a handful of bacteriophages in a single bacterium, Escherichia coli, under simple laboratory growth conditions became the springboard for molecular biology. Thanks to a revolutionary progress in DNA sequencing, bioinformatics and statistical techniques, microbiologists now study highly complex systems like the microbiome of human populations under various physiological and disease states. However, when going from a descriptive to a seemingly simple interventional stage in microbiome research, namely phage therapy of E. coli diarrhea in children, data limitations in the literature become apparent. Phage-E. coli interaction in the gut, their natural ecological niche, are fragmentary, E. coli etiology in childhood diarrhea is unclear and the gut microbiome analysis in diarrhea is just starting. From a failed clinical phage therapy trial two conclusions are drawn: on one side, we need an extension of the reductionist principle for E. coli phages into the gut of mice with controlled microbial colonization to understand their in vivo interaction as a basis for their medical application. On the other side, we need more exploratory clinical observations to confirm that infection concepts obtained from molecular pathogenesis work and animal models apply to the clinical reality.

Pseudomonas aeruginosa phages to disinfect plumbing materials and surfaces in contact with drinking and thermal water: feasibility study

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Pseudomonas aeruginosa is an opportunistic organism, especially problematic in hospital where it is responsible for almost 10 % of nosocomial infections behind Escherichia coli and Staphylococcus aureus. The water environment is considered as a key issue in P. aeruginosa colonisation of patients. Thus, many studies have attributed a major role to water network contamination in the incidence of patients' colonisation with P. aeruginosa especially in ICUs. In hospital water networks contaminations are often located in biofilm at terminal point-of-uses. Numerous infection control practitioners advocate regular cleaning, descaling and disinfecting of tap-water, sinks and U-bends to decrease cross-transmission via hospital water supplies. Thus, regular cleaning and disinfection of sinks and faucets using chlorination or thermal shock at 70°C for 30 min are used but these treatments may damage materials and numerous reports note the difficulty of reducing colonisation in tap water.

The aim of our study is to test the potential efficiency of phages as biocontrol agent against Pseudomonas aeruginosa in materials in contact with water, or removable outlets used in hospital but also in thermal institute. Our first approach is based on the application of several bacteriophages suspensions on planctonic and sessile cultures of Pseudomonas aeruginosa. We also developed a model of biofilm on different materials to test efficiency of bacteriophages on monospecies biofilm. First results highlight the specificity of some phages towards environmental strains of P. aeruginosa studied. Bacterial susceptibility depends on physiological cells state (included planktonic or sessile) but also on the concentration of phages used. While phages can specifically and rapidly reduce the populations of P. aeruginosa, resistance or adaptation can be observed in bacterial populations These phenomenons must be taken into account and further investigations are needed to optimize phages infection and to formulate bacteriophages combination to improve their efficiency.

In parallel, insights are needed with regard to the legal status of bacteriophages to develop disinfection applications. Even if bacteriophages can be used as an alternative to antibioresistance in human medicine or as a prophylaxis in farms with a specific status of drugs, the use of bacteriophages as disinfection treatment of surfaces and plumbing materials in contact with water may be approved by the biocides regulation.

Posters

Unraveling the dependence of SPP1 bacteriophage on molecular chaperones from the host Bacillus subtilis

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SPP1 is a lytic bacteriophage infecting the bacterium *Bacillus subtilis*. SPP1 is double strand DNA virus that belongs to the Siphoviridae family. It is a prototype of the ‘‘SPP1-like viruses", acting as a model system for viruses of Gram-positive bacteria.

During its infection cycle, SPP1 massively hijacks the host's resources, synthesizing 9 Mbp DNA and 150 000 polypeptide chains to produce 200 virions in 30 minutes, imposing a substantial challenge to the cell. Under normal conditions, molecular chaperones play an essential role in the folding of nascent chain polypeptides, refolding or degradation of misfolded and aggregated proteins, and other house-keeping and stress-related functions (1). Molecular chaperone GroEL and its cofactor GroES are essential for the folding of newly translated proteins under all growth conditions. DnaK also has a function in protein folding, assembly and export facilitation, and plays a role in prevention of protein aggregation. Together with the *dnaK* operon, the *groESL* operon from *B. subtilis* are known as the main chaperones of the cell. Several *E. coli* phages are known to use host GroEL for folding capsid proteins during morphogenesis, such as λ(2), T4(3) and RB49(4). Others, like the *Pseudomonas aeruginosa* phage EL, encode a GroEL ortholog, which presumably plays a role in endolysin folding during late stages of infection(5).

Because *groEL* is an essential molecular chaperone in the cell, its action mechanism is most likely essential in assisting the folding of viral particles. Thus, to assess SPP1 phage dependency on host molecular chaperones, we constructed isogenic knock-down (*groEL*) and knock-out (*dnaK*) strains. We then analyzed *B. subtilis* mutants during SPP1 infection, which was shown to depend on host GroEL, but not on DnaK. Fluorescence microscopy and qPCR analysis showed that DNA replication is not significantly affected, but phage capsid assembly is strongly impaired in the *groEL* knocked-down mutant.

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Nitric oxide-driven prophage maintenance involves unsuspected activity of NorV(W) reductase

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Bacterial genomes contain large amounts of prophages, either functional or defective. All these prophages contribute to bacterial genome evolution by providing an additional pool of genes that sometimes accommodate new properties to the host, a phenomenon called lysogenic conversion. Prophages usually highjack the host stress response signalisation to resume a lytic cycle when conditions become threatening for the host and therefore for the integrated prophage.

This work shows that nitric oxide serves as a maintenance signal and induces the production of NorV(W) reductase, which in turn prevents prophage induction independently of its usual activity. Surprisingly, nitric oxide, which is a potent nitrosative agent responsible for many cellular damages, does not promote prophage induction but rather maintains and counteracts the SOS-response outcome. Counteracting prophage induction makes particularly sense for enterobacteria when exposed to nitric oxide produced in the gut during inflammation or through their own anaerobic respiration.

Control and maintenance of prophages in Salmonella enterica

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Thanks to increasing genomic data, the importance of bacteriophages is highlighted in horizontal gene transfer (HGT). Bacterial genomes typically harbor multiple prophages carrying a wide range of genes that potentially provide new abilities. On one hand, these genetic elements may be selected because they confer an ecological advantage, such as new virulence factors or adaptive traits (1). On the other hand, uncontrolled expression of prophage genes could be detrimental. Thus, genome evolution by HGT requires a precise equilibrium between the repression and expression of newly acquired genes.

Salmonella enterica serovar Typhimurium is a primary enteric pathogen. Usually, *S. enterica* strains possess 4-5 functional prophages and prophage genes make up ca. 30 % of the pool of accessory genes (1). Of note, some prophages are known to provide virulence factors to the host (2). Our goal is to identify general strategies, other than phage repressor based, that are used to maintain prophage genes in the host genome. In this context, we have previously shown that the transcription terminator Rho is involved in prophage maintenance in *E. coli* (3).

A combination of targeted (mutants of major transcription regulators) and global (transcriptomics) approaches will help to identify: (i) which host factors are involved in prophage maintenance, and conversely, (ii) how prophage genes contribute to the metabolic versatility of *S. enterica*. So far, we assayed the effect of various host-encoded regulator mutants using multiplex and quantitative PCR. Preliminary results have shown that H-NS, a known negative regulator of horizontally acquired genes, controlled the excision of prophage Gifsy-1 in strain ST4/74. RNAseq results further suggest the implication of prophage genes in carbon metabolism and sugar uptake in strain ST14028s. Understanding how the host prevents prophage induction and how prophages modify the host physiology will give a clue on the mechanisms involved in cooptation and co-evolution of prophages within their host.

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New insights into A1, a multitasking pre-early protein of bacteriophage T5

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Immediately after delivery of their genome into the host, lytic phages defeat bacterial defences and hijack host cell machineries to establish a favourable environment for their multiplication. The early-expressed genes governing host takeover are highly diverse from one phage to another and most of them have no assigned function. They thus represent a pool of novel genes whose products potentially subvert or disrupt bacterial cell vital functions for phage profit. So far, however, their characterization is still at an early stage.

Bacteriophage T5 uses a unique two-step mechanism to deliver its 121 kb DNA into its host *Escherichia coli*[1]. At the onset of infection only 8 % of the genome enters the cell before the transfer temporarily stops. During the pause, the genes encoded by this DNA portion are expressed and their products lead to the host chromosome degradation, shut-off of the host gene expression and inactivation of the host defence systems (restriction/methylation, DNA repair)[2]. After a few minutes, T5 DNA transfer resumes allowing expression of the T5 middle and late genes and further phage productive growth. This original mechanism of DNA delivery facilitates the identification and functional characterization of the early genes responsible for host takeover, as they are clustered on the genome.

Two early proteins of T5, A1 and A2, are required for resuming the DNA transfer and additionally A1 is essential for host DNA degradation. We have demonstrated that purified A1 has an exo- and endo-nuclease activity *in vitro*. Moreover, ectopic expression of A1 in *E. coli* is sufficient to observe host DNA degradation by fluorescence microscopy. Taken together, our results indicate that A1 might be the so far elusive phage factor responsible for the massive host DNA digestion observed after the first-step transfer. Using bioinformatics tools, site-directed mutagenesis and complementation assays, we have identified some amino acids that are essential for infection and nuclease activity. An ongoing biochemical characterization could permit structure/function studies. Our findings raise the intriguing question of how A1 nuclease activity is coupled to the completion of phage DNA transfer.

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Sak4 of phage HK620 is an SSB-stimulated annealase that is involved in the lytic development of the phage

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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. Their characterization may allow finding ways to control the evolution of phages of interest. Moreover, it may help understanding how similar proteins work in other organisms. For instance, the family of phage Sak4 is composed of proteins similar to Rad51 paralogs acting in homologous recombination but for which the molecular mechanism remains poorly characterized.

Here, we report that Sak4 from phage HK620 that infects *Escherichia coli* TD2158 and encoded by *hkaL* is important for the life cycle of the phage as an *hkaL* mutant is impaired in lytic development. We also show that Sak4 is able to promote recombineering events *in vivo*, albeit at a lower extent compared to the Redβ protein. To characterize Sak4 at the molecular level, we undertook its biochemical characterization. Sak4 is an ATPase that binds single-strand DNA in an ATP-dependent way, its ATPase activity being stimulated in the presence of ssDNA. Sak4 performs annealing of complementary ssDNA with a low efficiency. The weakness of Sak4 annealase activity can be explained by its dependence on another protein partner. Indeed, 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*. *In vitro*, the binding of Sak4 to ssDNA is stimulated by its cognate SSB, as well as its annealase activity. Interestingly, these positive effects are strictly dependent on the C-terminal 6 amino acids of its cognate SSB, residues that are known to be involved in protein-protein interaction in other bacterial SSBs. Finally, the stimulation of the ATPase activity of Sak4 is decreased in the presence of ssDNA when its cognate SSB is present. We propose that the phage SSB facilitates the recruitment of Sak4 on DNA via a direct interaction. This recruitment probably stabilizes Sak4 on DNA, a prerequisite to an efficient annealase activity of this recombinase. To date, Sak4 of phage HK620 is the first described single-strand annealing protein that uses an SSB to enhance its activity.

Hidden phage Vp16 PDF features are essential for deformylase activity

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Prokaryotic proteins must be deformylated before the removal of their first methionine. Peptide deformylase (PDF) is indispensable and guarantees this mechanism. Recent genome sequencing studies highlighted over 2x104 putative peptide deformylase sequences. Furthermore, unpredicted modified bacterial PDF genes have been retrieved from many viruses. Sequence comparisons with other known PDFs reveal that viral PDFs are devoid of the key ribosome-interacting C-terminal region. Little is known regarding these viral PDFs, including the capacity of the corresponding encoded proteins to ensure deformylase activity. We provide here the first evidence that viral PDFs, including the shortest PDF identified to date, *Vp*16 PDF, display deformylase activity *in vivo*. Large scale N-terminomics characterization reveals that *Vp*16 PDF has substrate specificity similar to that of other bacterial PDFs. However, our integrated biophysical and biochemical approaches also reveal hidden and unique functions of the unusual C-terminus. The high-resolution crystal structures of *Vp*16 PDF, free or bound to the potent inhibitor actinonin, reveal a classical PDF fold and also an unexpected crucial role for the ultimate residue tethering the active site. Our study underscores the structural and molecular characteristics of the unusual C-terminal Ile residue that sustains deformylase activity in the absence of the otherwise indispensable C-terminal domain.

Deciphering phage trajectories with interferometric microscope

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We recently developed a very simple new optical microscope, which detects nanoparticles including viruses. The measurement is based on the determination of the scattering signal of each particle and its Brownian motion as the microscope records a film of 200 frames over a few seconds (typically around 2 seconds with the 150Hz New Focus CMOS camera). With these two measurements (scattering signal and particle tracking) we compute the particle diameters.

Thanks to the frequency of our camera, we were able to observe that T4 phage instead of Brownian trajectories exhibits peculiar trajectories. We characterize the T4 displacements as well as classical Brownian trajectories of other viruses by two methods: one based on the positions of the particles along given trajectories and the other, which takes in account the time parameter. We will present recent results and hypothesize on these unusual displacements.

Genome-wide CRISPRi screen reveals bacterial requirements for phage infection

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Phage therapy and phage-derived technologies have gained a new interest in the past decades through the emergence of antibiotic-resistant bacteria. However, phage-host interactions remain poorly characterized due to the lack of fast and cost-effective screening methods. Here, we developed a high-throughput CRISPR-based screening method to detect bacterial genes involved in phage infection. This genome-wide method was used to compare phage receptors and lipopolysaccharide (LPS) requirements between different coliphages and highlighted the importance of LPS in the adhesion of phage 186. A two-step experimental setup then enabled to discriminate genes required for host death from genes required for the production of functional progeny particles. The screening process highlighted the importance of essential genes and detected major host genes previously associated with lambda phage infection while unveiling new candidates. This study emphasizes the ability of CRISPR/dCas9 screens and sgRNA libraries to detect host factors for phage infection and provides directions to quickly apply the method to other host-phage systems.

Enological environment as a source of a novel tectivirus

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Acetic acid bacteria (AAB)represent spoilage microorganisms during winemaking, mainly because they are able to produce ethyl alcohol and transform it into acetic acid. The bacteriophage GC1 infects the AAB species *Gluconobacter cerinus* and has been isolated from a juice sample collected during dry white winemaking. Transmission electron microscopy showed tail-less icosahedral particles. Genome sequencing revealed a linear double-stranded DNA (16,523 base pairs). GC1 genome contains terminal inverted repeats and carries 36 open reading frames, only five of which could be functionally annotated. These elements highlighted that GC1 is a novel member of the *Tectiviridae* family. Further investigation revealed that the bacteriophage has limited similarity to other tectiviruses and is a divergent member of the genus *Alphatectivirus*. GC1 is the first tectivirus infecting alphaproteobacterial host and is the first temperate alphatectivirus. Furthermore, GC1 helps to bridge the gap in the sequence space between alphatectiviruses and betatectiviruses.

Survey on prophages infecting Weissella cibaria and Weissella confusa

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Lactic Acid Bacteria (LAB) infection by bacteriophages could be responsible of impaired food fermentations and/or alterations of product quality. However, there are few studies concerning phages in fermented products other than dairy foodstuffs. *Weissella cibaria* and *W. confusa* belonging to *Leuconostocaceae* family (also including *Leuconostoc*, *Oenococcus* and *Fructobacillus* genera) have been isolated from a wide range of habitats, and are known to be involved in several traditional food fermentations such as starchy or cereal-based products, fruits and vegetables, meat and fish products. Moreover, *W. confusa* and *W. cibaria* strains are recognized to produce copious amounts of oligosaccharides and exopolysaccharides with special interest for applications such as texturizing agents and prebiotics (Bounaix et al. 2010). It is thus important to increase the knowledge on phages infecting these two species.

No phage has been yet described to infect *Weissella confusa* and only two phages infecting *Weissella cibaria* were currently described, both belonging to the family of *Podoviridae*: φYS61 has been isolated from kimchi and sequenced (Kleppen et al., 2012), and φ22 was isolated from Nham (Pringsulaka et al., 2011). Besides, several phages infecting *Weissella cibaria* were isolated from commercial cucumber fermentation (Lu et al., 2012) and one phage infecting *Weissella* sp. has been isolated from sauerkraut fermentation (Lu et al., 2003).

The aim of the present study was to investigate the presence of prophages in *W. cibaria* and *W. confusa* strains previously isolated from French sourdoughs (Robert et al., 2009) and from other biotopes. First, prophage excision was achieved with mitomycin C induction and led to observe bacterial lysis for all the isolates. Unfortunately, permissive strain(s) could not be detected over various LAB strains tested. Phage DNA was thus purified from mitomycin-treated cultures and phage genomes were compared using conventional restriction profile, and analysis of PCR products obtained using (GTG)5 primers, as a new phage DNA comparative technique.

Overall, the results of this study clearly demonstrate the prevalence and diversity of prophages in the genome of *W. cibaria* and *W. confusa* strains.

This study is part of work program ANR LYSOPLUS.

Phages infecting Leuconostoc mesenteroides in dairy products

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Lactic acid bacteria (LAB) are used in food industries for fermented products, for instance dairy products such as cheeses. Among LAB, *Lactococcus lactis*, *Streptococcus thermophilus* and *Lactobacillus* species are the most frequently used; but *Leuconostoc* are also recurrently found inside cheeses where they play a crucial role in aroma production. Besides, they contribute to the formation of openings in some soft, semi-hard and blue-veined cheeses. Many food makers experienced acidification and sensorial defaults, which can be related to phage attacks towards *Leuconostocs*. However knowledge about presence and diversity of *Leuconostoc* phages is still lacking. Phage attacks can result from lytic or temperate phages, but also from autoinduction of integrated-bacterial genome prophage. It leads to lysis of the bacterial cell and to fermentation defaults. At present, PCR methods were only developed to detect lytic phages in dairy products (Ali et al., 2013).

Our main objective was to increase knowledge about *Leuconostoc mesenteroides* phages, firstly by testing the presence of prophages in the bacterial genome and their possible induction; then by assessing the presence of free replicating phages in different dairy products. Prophages were induced by stressful agents. Phage DNA were then extracted and digested with restriction enzymes to allow them to be compared. From now on, we intend to detect free replicating phages from dairy products. During the course of this project, we intend to develop new technics to detect *Leuconostoc* temperate phages in products.

This study is part of work program ANR LYSOPLUS.

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PhagesCollect : an updated and functional collection of dairy bacteriophages

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Lactic acid bacteria are an heterogeneous group of Gram positive, non-spore-forming bacteria with a rod-shaped or coccoid morphology. The ability of lactic acid bacteria to produce lactic acid is commonly used for the production of fermented food products, in particular for the fabrication of dairy products. *Lactococcus lactis* and *Streptococcus thermophilus* are the most intensely employed starter bacteria in dairy fermentation. The use of such starters in modern processes involves intensive mass production, thereby increasing the risk of bacteriophage infection. Bacteriophage infection in dairy-cheese may lead to unacceptably low lactic acid and flavor compounds production, resulting in high economic losses.

The aim of the PhageCollect project was to isolate and study the diversity of bacteriophages infecting *S. thermophilus* and *L. lactis* currently circulating in french dairy-cheese plants as well as to build and sustain an updated and functional collection of bacteriophages of french dairy origin.

139 samples of whey were collected from different French geographic area, and from different dairy products. Samples were tested for the presence of bacteriophages. Results showed that 40,3% and 84,3% of whey samples contained at least one bacteriophage strain when using 5 *S. thermophilus* and 12 *L. lactis* propagations strains. On the one hand, 86 positive reactions were observed regarding all whey samples and *S. thermophilus* propagation strain couples. On the other hand, 125 positive reactions were observed regarding all whey samples and *L. lactis* propagation strain couples. Bacteriophages were purified and amplified using appropriate bacterial strains. Bacteriophages were typed using either comparison of VR2 sequence (a variable region of the antireceptor gene) for *S. thermophilus* bacteriophages, or comparison of restriction profiles for *L. lactis* bacteriophages. Diversity study revealed the presence of 27 *S. thermophilus*-phage groups while bacteriophages infecting *L. lactis* were clustered in 42 different groups.

In conclusion, bacteriophages infecting lactic acid bacteria are widespread in dairy cheese plants and exhibit high genetic diversity. This diversity seems to be higher for *L. lactis* bacteriophages, most likely because 3 subspecies are usually used in dairy transformation (*cremoris*, *lactis* and *diacetylactis* subspecies). Bacteriophages isolated in this project were included into the open collection of the French Dairy Interbranch Organization (CNIEL). This open access phage library and associated information will provide researchers and producers with a useful tool to prevent and/or increase responsiveness against phage risk.

Genetic characterization of two siphoviridae targeting marine magnetotactic bacteria from the Mediterranean Sea

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Horizontal gene transfers (HGT) represent for microorganisms tremendous opportunities to improve their fitness in ever shifting and competing environments or acquire new functions and colonize new ecological niches. Bacteriophages are efficient mediators of such HGT through more or less targeted infection, bacterial gene carryover and eventual integration within their host's genome. Bacterial magnetotaxis - the ability to orientate and swim along the geomagnetic field - is supported by a set of dedicated genes that spread among phylogenetically diverse aquatic microorganisms, the magnetotactic bacteria (MTB). Vertical inheritance but also HGT have been previously put forward to account for the phylogenetic diversity of MTB and their geographic ubiquity. We aim at investigating the role of bacteriophages in the diffusion of magnetotaxis in aquatic environments by matching MTB strains and viruses isolated from the same spot in the Mediterranean Sea at 7.5 m depth off the coast of Marseille. We report here the isolation and genomic characterization of two *siphoviridae* propagated on PR1, PR2 and PR3 MTB indigenous strains.

Gut Phageome: how to extract and analyze phage DNA

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The microbiota of the human gut called nowadays « the second brain » is a very complex and rich community composed of bacteria, viruses, archaea, protozoa and fungi, coexisting in equilibrium. The study of the dynamics and stability of the human gut microbiota is essential to understand their role in disease, diagnosis, treatment and prevention. Bacteria and their viruses (bacteriophages) are the most important members of this community. Gut bacteria have been very thoroughly studied in the last few years, partly because their species can be identified by relatively inexpensive 16S sequencing. But, what about the gut bacteriophage where no equivalent marker exists? Current methodologies for extracting the phages DNA from the feces, for sequencing them and analyzing with bio-informatics tools are very heterogeneous making comparisons between studies difficult. The main objective of our work is to define a specific methodology for each step: extracting, sequencing and analyzing phage DNA from feces.

After the comparison of five different techniques to extract phages from feces, the PEG concentration methods showed the highest number and diversity of phage sequences with also the best ratio cost/efficiency. We also tested the influence of using a polymerase (phi 29) and showed that its use severely decreases the sampled genetic diversity and shows poor reproducibility. Finally, we tested a number of ways of assembling and annotating the sequences obtained from three healthy volunteers and propose a set of methods that take advantage from published data on the genomes of phages and prophages.

Phages application to control Pseudomonas aeruginosa contaminations from terminal water points of use

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Objectives:

Pseudomonas aeruginosa belongs to the group of multidrugs resistant bacteria. This microorganism is an opportunistic pathogen present in some water network. Contaminations are often located at terminal point-of-use, especially in hospital and thermal institutes giving rise to sanitary and economical risks. While, different technologies and processes are routinely used to treat water outlets, some contaminations remained persistent due to the formation of biofilm. One reason for this, is the penetration barrier provided by biofilms exopolymers accumulation around cells, preventing biocides action. In this context, the research challenge is focused on the development of an alternative treatment which could be, in addition to existing methods, participate to *P. aeruginosa* biofilms elimination of this kind of surfaces. Bacteriophages are exclusively natural predator viruses of bacteria. They are ubiquitous in the environment allowing us to have a wide diversity available. Goals of this study will be to test the potential efficiency of phages as biocontrol agent against *Pseudomonas aeruginosa*.

Materials and Methods:

Seven *Pseudomonas aeruginosa* strains included the reference strain PA01 and 3 environmental strains were used to studied activity of 9 bacteriophages. 2 phages have been isolated from the environment with double layer plate method and 7 others have been previously described. Spot tests have been used to screen susceptible bacteria and choose couples (phages/bactéries) will be studied. Bacterial strains were cultivated in minimum medium and efficiency of different phages MOI (0.1, 1, and 10) was studied on exponential culture by optical density (OD 600nm) analysis and later by vPCR.

Results and Perspectives:

Bacteria susceptibility depends of their physiological cells state (planktonic or sessile) but also of the MOI used. This study highlights the specificity of 3 phages towards three environmental strains of *P. aeruginosa* studied as well as PAO1 susceptible ref strain. Although two of them are susceptible to only one phage, the third environmental strain is sensitive to all three phages. In these cases, results show until 90% of population decrease. Among this three phages, one belongs to *Podoviridae* family, another to *Myoviridae* and last has to be characterized by MET. In this way, further investigations are needed to better understand phages infection of these strains. Next step will be to test phages activity on biofilm of this environmental strain on stainless steel coupon.

Efficacy of coliphages to prevent chicken embryo mortality

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Avian colibacillosis, induced by specific *Escherichia coli* strains, is the major bacterial disease in poultry and is mainly treated by antibiotics. Increasing resistance to different antibiotic classes and restrictions on the use of antibiotics by the European Union led to the search for antibiotics alternative such as bacteriophage therapy. We have isolated and characterized various coliphages that are promising biocontrol agent. Among those phages, several are highly related to phAPEC8 and others to phage phi92. When mixing *in vitro* two phAPEC8-related phages, we observed the appearance of a recombinant phage, which exhibited a broader host range and a higher lytic activity compared to parental phages. We thus tested in a chicken embryo lethality assay the therapeutic potential of this recombinant phage alone or in combination with and phi92-related phage. We observed that the combination of both phages allowed 100% of chicken embryo to survive to an infection by an avian pathogenic strain. This result strengthens the effective potential of phages to control avian colibacillosis.

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