

Colloque

Interactions phages-bactéries : du fondamental à l'appliqué

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Présentation orales

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Résumés PRESENTATIONS ORALES

(1) Molecular mechanisms of viral DNA packaging: recognition and cleavage of the bacteriophage SPP1 pac sequence during packaging initiation

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Specific recognition of the viral genome is an essential step in virus assembly. Packaging of the bacteriophage SPP1 DNA is initiated by recognition of the sequence pac within the viral DNA concatemer by the small subunit of the terminase (gp1, TerS). Pac is subsequently cut by the terminase large subunit (gp2, TerL). This cleavage generates a DNA free end that is the starting point for the unidirectional packaging reaction. This work aims to elucidate the mechanism underlying pac recognition and cleavage by the terminase. We present a study of the role of two different pac sequence sub-domains: pacC (cleavage region) and pacR (right region of the pac sequence that is encapsidated). Using a plasmid system that co-expresses pac and the two subunits of the terminase in *Bacillus subtilis*, the SPP1 host, we show that the pac cut doesn't require a specific sequence in pacC and that mutations on pacR affect the reaction. Altogether our data support a model for viral DNA packaging initiation in which binding of the TerS N-terminal domain to a specific sequence of pacR creates a structural context to recruit the sequence-independent nuclease TerL, and direct it to cleave exclusively at pacC.

(2) Insights into the architecture and structure of phage T5 tail

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The tail of Caudovirales bacteriophages serves as an adsorption device, a host cell-wall perforating machine and a genome delivery pathway. In Siphoviridae, the tail is long and flexible. We combined a proteomic analysis of siphophage T5 particles with a bioinformatic study and electron microscopic immunolocalisation to assign function to the structural proteins of the tail of this siphophage infecting *E. coli*. Infection of *E. coli* cells by T5 is triggered by the irreversible binding of pb5, at the tip of the straight fibre, to the outer membrane transporter FhuA. Binding of pb5 to FhuA induces opening of the capsid and perforation of the host cell wall. An elegant Small Angle Neutron Scattering study allowed to investigate the conformational changes occurring upon interaction of the two proteins. Furthermore, we structurally characterised the protein pb9 of T5, which reveals remarkable structural similarity with known Distal tail proteins (Dit). The modular structure of the Dit protein maintains the basic building block that would be conserved among all siphophages, combining it with a more divergent domain that might serve specific host adhesion properties.

(3) Assembly and Maturation of Bacteriophage T5 capsid

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Capsids of dsDNA bacteriophages are extraordinarily robust macromolecular assemblies capable of withstanding the strong internal pressure generated by the tightly packed DNA they contain. Their assembly is a regulated stepwise process exhibiting conserved features in all tailed bacteriophages. An empty icosahedral procapsid is initially assembled from several hundred copies of a major coat protein. A dodecameric portal protein occupies a unique vertex and forms a gate through which DNA is packaged. The terminase drives DNA translocation into the procapsid, a process accompanied by the expansion of the capsid shell, which involves a structural rearrangement of the head protein subunits. Accessory proteins decorate the surface of the DNA-filled capsid and head completion proteins seal the portal gate and provide the binding site for the tail. Phage T5 differs from other well-studied siphophages by the large size of its capsid and genome (121 kbp). We have established the molecular basis of the assembly pathway of T5 capsid and shown that several steps can be reproduced in vitro: assembly of the procapsid from its elementary components, capsid expansion and decoration. These features make T5 a very attractive system for investigating the maturation of dsDNA bacteriophage capsids and for providing functionalized nanoshells.

(4) Development of an interferometric microscope to sort viruses: tests and applications

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We developed a new optical method to detect viruses and other nanoparticles. The method uses the scattered signal and the Brownian motion of individual nanoparticles to sort them. The method has been validated with 50 nm diameter beads and purified viruses of different size and shape. The method is suitable whatever genetic material present into the virus particles (RNA or DNA, single or double strand). With this first setup we have been able to detect viruses as small as 30 nm in diameter. Analysis of Brownian motion trajectories helped us to define a specific signature for myoviruses. Computation of diameters obtained by the two methods (scattering signal and Brownian motion) helped in discrimination between viruses and vesicles. We were able to sort particles from complex environments such as samples from the gut and

oceans (TARA-oceans). These data will be presented and the advantage of using this method coupled with metagenomic analysis.

(5) Phage adsorption to bacteria in the light of the electrostatics: a case study using *E. coli*, T2 and flow cytometry.

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The addition of sodium chloride to freshwater or diluted minimal salt medium increases the adsorption of T2 phages on *Escherichia coli*. For the first time the adsorption in diluted minimal salt medium was measured by counting unadsorbed phages (i.e. free particles) using flow cytometry, allowing a gentle separation between adsorbed and unadsorbed phages. Flow cytometry was able to detect weakly adsorbed phage that remained undetected using classical centrifugation-based methods and this allowed us to show that increasing ionic strength enhances the phage adsorption to its bacterial host with an extremely low detection limit. A key result was that the adsorption in high ionic strength (i.e. 100 mM) reached $4.5 \pm 0.1 \times 10^{-5}$ mL/min which is 1400 fold higher than previously reported values. In order to understand the mechanism underpinning such a weak phage adsorption, the zeta potentials and the diffusion coefficient of the particles were measured by dynamic light scattering. The bacterial cells and the phages had zeta potentials between -60 mV and -10 mV and -30 mV and -10 mV, respectively. The diffusion coefficient of the phage was $2.8 \pm 0.4 \times 10^{-12}$ m² s⁻¹ corresponding to a hydrodynamic radius of 104 ± 15 nm. However significant adsorption occurs in conditions where the DLVO theory predicts that minimal encounter, suggesting that forces other than electrostatic repulsion and Van der Waals interaction (e.g. potential impurities, particle shape and other biological characteristics) are likely to interplay.

(6) The chromosomal accommodation and domestication of temperate phages in *Escherichia coli*

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Prokaryotes are constantly being infected by large mobile genetic elements such as temperate phages. Once integrated, these prophages are major contributors to the diversity of bacterial gene repertoires. The fitness of these elements is tightly linked with the evolutionary success of the host. This leads to selection against disruptive effects their integration might have on the organization and structure of the

chromosome. Seamless genetic accommodation of these elements also involves silencing infectious mechanisms and expressing functions adaptive to the host. Ironically, these characteristics favor the host ability to domesticate such element. Recent data obtained using only computational approaches, suggest that the domestication of temperate phages might be frequent in *E. coli*. Importantly, it might affect the evolution of chromosome organization and drive the diversification of social traits.

(7) Vaccination prevents lysogenic conversion and the evolution of *Salmonella Typhimurium* virulence

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Imperfect vaccines, i.e., vaccines that are protective, yet allow host colonization and transmission of pathogens, could influence virulence evolution. The direction towards attenuated or increased virulence depends on the mechanisms that underlie the host-pathogen interaction and remains difficult to foresee. This question is rarely experimentally addressed and consequences of imperfect vaccination on enteropathogens evolution are unknown. We have developed a protocol for imperfect vaccination which renders mice almost insensitive to intestinal colonization by *Salmonella Typhimurium* (S. Tm). In this model, fully virulent strains of S. Tm can reach maximal intestinal carrying capacity but are unable to trigger disease. We observed that vaccination drastically limits the transmission of the temperate SopE Φ -bacteriophage between two strains of S. Tm. Although SopE Φ does not affect the fitness of the recipient strain, lysogenized recipients reach 25% of the total population after three days in naïve mice. In protected mice, the lysogens remain undetectable. We have found that intestinal inflammation is essential to trigger SOS response mediated lytic cycle and phage transfer. SopE Φ encodes an effector protein secreted via a Type III secretion system and involved in host cell invasion. Therefore, our results reveal that vaccination against enteropathogens prevents virulence factors exchanges via lysogenic conversion.

(8) Role of prophages in intestinal microbiota stability

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Le microbiote intestinal est composé de plus de 500 espèces bactériennes différentes, majoritairement des Firmicutes et Bacteroidetes. Des modifications de la composition du microbiote intestinal (dysbiose) sont associées à certaines maladies humaines, en particulier les Maladies Inflammatoires Chroniques de l'Intestin (MICI). Le rôle des phages dans les dysbioses est encore très peu exploré. La majorité des

bactéries du microbiote intestinal séquencées abritent un ou plusieurs prophages. Nous faisons l'hypothèse que les prophages participeraient à la dysbiose en agissant comme des bombes à retardement moléculaire sur des souches clés pour l'équilibre du microbiote intestinal. Ma thèse porte sur l'étude de l'induction des prophages dans un microbiote intestinal simplifié de souris composé de 5 souches. Dans un premier temps, par approche bioinformatique, j'ai prédit quatorze prophages dans quatre des cinq souches. L'ADN de neuf des prophages a été détecté dans des virions par PCR quantitative (qPCR) et séquençage. Différents signaux inducteurs ont été testés sur les souches pour déterminer les conditions d'induction des prophages. Par ailleurs nous avons mis au point un protocole pour extraire les phages des fèces de souris et les quantifier en qPCR. Cela permettra de mesurer le niveau d'induction des prophages dans le tube digestif des souris, notamment en condition inflammatoire .

(9) Carriage of λ latent virus is costly for its bacterial host due to frequent reactivation caused by DNA damage in the mouse intestine

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Prophages, the latent form of temperate bacteriophages, are omnipresent in bacterial genomes. Whereas these prophages are generally considered to increase the fitness of their hosts, *in vivo* studies demonstrating such effects are scarce. We performed competitions between two *Escherichia coli* strains, isogenic except for the presence of λ prophage, to determine the impact for bacteria of prophage carriage and estimate the parameters ruling phage-bacteria interactions in monoxenic mice gut. We show that phage infection of the bacterial strain devoid of the prophage resulted in 70% of times in lysis. Thanks to this killing of susceptible bacteria, the initial phage carrier strain, immune to infection, increase in proportion. However, in the absence of susceptible competitors, prophage induction in as much as 1% of bacteria induced a significant fitness cost for their carriers. This high prophage induction rate detected reveals DNA damage-mediated SOS response in the mouse intestine. Since λ propagated also very efficiently by lysogenization (30% of infections), it enabled rare events of phage gene capture by homologous recombination to occur in the intestine. We conclude that the mammalian gut, the most densely populated bacterial ecosystem on earth, fosters bacterial evolution through high temperate phage activity.

(10) Aligning the unalignable: bacteriophage whole genome alignments

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In recent years, many studies focussed on the description and comparison of large sets of related bacteriophage genomes. Due to the peculiar mosaic structure of these genomes, few informative approaches for comparing whole genomes exist: dot plots diagrams give a mostly qualitative assessment of the similarity/dissimilarity between two or more genomes, and clustering techniques are used to classify genomes. Multiple alignments are conspicuously absent from this scene. Indeed, whole genome aligners interpret lack of similarity between sequences as an indication of rearrangements, insertions, or losses. This behavior makes them ill-prepared to align bacteriophage genomes, where even closely related strains can accomplish the same biological function with highly dissimilar sequences. We propose a multiple alignment strategy that exploits functional collinearity shared by related strains of bacteriophages. As classical alignments do, the computed alignments can be used to predict that genes have the same biological function, even in the absence of detectable similarity. The Alpha aligner implements these ideas and is used to produce several examples of alignments of *Staphylococcus aureus* and *Mycobacterium bacteriophages*, involving up to 29 genomes.

(11) Phagonaute: an interface to navigate accross phage modules and detect distant homologies

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Distant homology search tools such as HHpred are a great help to predict phage protein functions, but as they rely on databases of pre-computed protein profiles that are not dedicated to phages, they can lack sensitivity. We developped a database storing the results of HHsearch phage proteins comparisons, among the 82156 proteins encoded by 952 phages. We then built a publicly available interface named "Phagonaute", to help predict phage protein functions. Distant homology results are displayed together with their genetic context, to secure the prediction. Each phage protein profile is also compared to Pfam, giving an orthogonal functional prediction. The phage community is now welcome to dig into the treasure, and launch appropriate experimental validations of the predictions offered by Phagonaute. An example of function discovery in the field of phage recombination will be presented.

(12) Molecular characterization of two new bacteriophages infecting *Pseudomonas aeruginosa*

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Virulent bacteriophages infect and lyse bacteria, including multi-drug resistant bacteria. We previously isolated new bacteriophages that efficiently cured lung infections caused by *Pseudomonas aeruginosa* in a mouse model. To understand how these bacteriophages efficiently infect their host, we initiated molecular studies of two of them (PAK_P3 and PAK_P4). We first determined their infection parameters and transcriptomic profiles using RNA-Seq. We evidenced a host transcriptomic response as well as a temporal regulation of viral gene expression along with other unexpected features, such as antisense transcription and expression of small ncRNAs. Further characterization of phage-host interactions was initiated using a transposon mutant library and led to identify host genes required for phage infection. Amongst conserved viral ORFs of unknown functions, three coding sequences were found to alter bacterial growth (of both *P. aeruginosa* and *Escherichia coli*) when expressed ectopically. Using a bacterial two-hybrid system, we are currently seeking partners of one of these proteins (gp92). Preliminary data reveal that gp92 might target key host regulatory pathways through interactions with inner membrane host proteins such as MucA, an anti- σ E factor. Overall, our molecular studies of novel therapeutic bacteriophages reveal new facets of bacteriophage infection and host response mechanisms.

(13) A new screen to detect host factors involved in prophage maintenance

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Temperate phages have the ability to integrate their genome into the host chromosome, a process called lysogeny. During evolution, some prophage genes can be lost, especially those coding for lytic cycle capacity. While some of these defective prophages are perfectly competent for excision, they are maintained in bacterial genomes, suggesting a selective pressure to keep them. This is the case for our model system, the defective prophage KpIE1 in *Escherichia coli* K12. To study prophage maintenance in Enterobacteria genomes, I developed a genetic screen to identify host factors involved in prophage maintenance. To detect KpIE1 maintenance, I inserted a fluorescent cassette within the prophage causing fluorescent colonies. Under excision conditions, there are no more fluorescent cells because KpIE1 cannot replicate and is thus lost by clonal dilution. The screen consists in transforming the reporter strain with a plasmid library covering the host genome. Under excision conditions, plasmids that allowed fluorescence emission,

and thus prophage maintenance, were selected. First results show the implication of genes coding for proteins involved in detoxification of nitric oxide (NO) in KpIE1 and HK620 prophage maintenance. Current work aims at characterizing the link between NO detoxification and prophage maintenance and by extension horizontal gene transfer.

(14) Resident prophages under host control

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Temperate phages are able to integrate their genome into the host and replicate passively through a lysogenic state. Hosts frequently benefit from such a massive gene acquisition through lysogenic conversion, and as prophages may be beneficial to their hosts, we hypothesized that hosts adapted strategies to maintain that gene source. Our results show that lysogeny maintenance of a class of prophages, which all share the same unusual genetic organization, are controlled by the transcription termination factor Rho. Rho is thus not only involved in horizontally acquired gene silencing but also in prophage maintenance, which can be seen as an adaptation of the host to maintain prophage genes. For these prophages, whether defective or functional, their induction by the inactivation of Rho, involves a new pathway of lysogeny escape, which is independent of the classical the SOS response pathway. Other regulatory interactions are under study in various Enterobacteria species to understand how prophage genes are integrated into the host regulatory network. These newly characterized interactions reflect the co-evolution of host and viruses, allowing the acquisition of genes, and thus new properties, via horizontal transfer, while controlling the expression of deleterious genes.

(15) Mutualistic interactions between *Pseudomonas aeruginosa* and its bacteriophages

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Biofilm, bacterial motility and mucoidy are bacterial characteristics that make *P. aeruginosa* a pathogen. If phage predation selects for variants with alterations in any of the genes that are involved in biogenesis or regulation of these virulence determinants, the resulting phage-resistant variants could potentially exhibit altered levels of virulence, in a beneficial or detrimental way. The purpose of our work is to

investigate the mechanisms and frequency of resistance acquisition in response to infection by cocktails of virulent phages belonging to different genera. Changes in bacterial phenotypic traits and virulence determinants are also analysed. Phase variations mutations in genes involved in the LPS production, O-antigen synthesis and in the alginate regulation were frequently identified. Cross resistance was systematically observed for phages using LPS as a receptor. Pseudolysogeny was a frequent outcome of the infection, and in some cases was maintained for more than 10 colony purification steps, allowing the emergence of new mutations. The use of cocktails did not lower significantly the frequency of phage-resistance and in addition we observe that pseudolysogeny is a major factor in selection of mutations.

(16) Phage and antibiotic combined effects on bacterial evolution

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Antibiotic resistant bacterial infections are a major concern to public health. Phage therapy has been proposed as a promising alternative to antibiotics, but an increasing number of studies suggest that both of these antimicrobial agents in combination are more effective in controlling pathogenic bacteria than either alone. We will present experimental results on the effects of antibiotics on the co-evolutionary dynamics of lytic phages and the bacterium *Pseudomonas aeruginosa*. We will show that combinations of phages and antibiotics can result in many positive outcomes, their main novel feature being synergistic effects in pathogen density control, whilst minimizing the evolution of resistance. In addition, we identify compelling challenges for the realistic application of combined phage-antibiotic therapy.

(17) Hot spots are cold spots: bacteria-phage coevolution in variable temperature environments

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Coevolution between hosts and parasites drive species diversity and parasite virulence evolution. However, antagonistically interacting species do not necessarily share the same environmental optima, which may have important consequences for the strength of coevolution when populations are exposed to variable environments. We used microcosm populations of the bacteria *Pseudomonas fluorescens* infected with the phage phi2 to investigate how the frequency of fluctuations in temperature

between 28°C, permissive for both players, and 32°C a temperature stressful for phage, affects coevolution. Phage density declined rapidly during periods at 32°C, but recovered quickly once being returned to 28°C. Host-parasite coevolution was characterised by arms race dynamics (ARD) under high frequency temperature fluctuations and at 28°C constant. Coevolution did not occur during periods at 32°C causing deviations from ARD when populations experienced lower frequency temperature fluctuations. Suspended coevolution during extended periods at higher temperatures was mostly due to direct effects of temperature on coevolution, possibly due to the modification of bacteria surface proteins preventing phage adsorption. Thus environmental temperature fluctuations can create a temporal refuge for bacteria providing respite from coevolutionary dynamics, even becoming cured of infection if periods at 32°C are sufficiently long.

(18) Evolutionary epidemiology theory of bacteriophages

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Theory predicts that pathogens that 'keep their host alive' can sometimes outcompete virulent and more transmissible pathogens in times when transmission to new susceptible hosts is unlikely. We model this transient benefit for virulence and predict both the epidemiology and the evolution of pathogens during an epidemic. To put these predictions to the test we monitor the competition of the temperate bacterial virus λ and its virulent mutant λ_{cl857} in experimental epidemics. In a second experiment we used the same experimental system to study the effect of spatial structure on virulence evolution. Theory predicts that spatial structure may limit access to susceptible hosts and favor less virulent strategies. Our experimental results agree remarkably well with our theoretical predictions. This demonstrates the ability of evolutionary epidemiology to predict selection for virulence in ongoing epidemics.

(19) Etat des lieux de la phagothérapie en France

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CH Villeneuve St Georges.

Il y a près d'un siècle, la phagothérapie a été initiée en France puis employée dans le monde pendant de plusieurs décennies. Mais si elle a été délaissée au profit de l'antibiothérapie chimique, un renouveau se présente aujourd'hui pour l'utilisation des bactériophages dans de nombreux domaines. En particulier, certains médecins qui sont confrontés au problème de l'évolution des résistances bactériennes aux antibiotiques envisagent leur utilisation. Même s'il existe encore des sceptiques

(dubitatifs) dans le monde médical, la phagothérapie est de plus en plus acceptée, voire souhaitée, dans certaines spécialités. Pour parvenir à sa réintroduction, un certain nombre d'initiatives émergent. Les problèmes à résoudre sont multiples et concernent des disciplines variées. Pour les résoudre, il faudra peut-être envisager un concept nouveau.

(20) World premiere: multicenter clinical study of phage therapy in burn wounds Launch of the clinical trial PHAGOBURN

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Pherecydes Pharma developed two phage cocktails authorized to be used in the first international clinical study named PhagoBurn to evaluate the efficacy and safety of phage therapy to treat burned skin infections with *E. coli* (PP0121) and *P. aeruginosa* (PP1131). Pherecydes Pharma is also developing new products.

(21) Towards an adequate regulatory framework for bacteriophage therapy

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Resistance of bacteria against antibiotics just keeps growing. Each year, 25.000 European citizens die because of this. Industry's pipeline does not contain much new antibiotics. Before the pharmaceutical development of antibiotics, e.g. natural bacteriophages (= bacterial viruses) were commercialised and used to kill pathogenic bacteria. This therapeutic application of natural bacteriophages was called "bacteriophage-therapy". Today, countries like Poland, Georgia and Russia are still practising bacteriophage-therapy. The European Union and "modern" medicine as a whole needs an urgent return of bacteriophage-therapy as part of its armamentarium to fight bacterial resistance to antibiotics. A European regulatory frame to make a smooth and (tailored) qualitative return of bacteriophage therapy possible is actually lacking. The presentation will propose a European regulatory frame that could fit the re-introduction of bacteriophage therapy without losing sight of the safety, quality and efficacy aspects related to the therapeutic use of bacteriophages.

(22) An ecology and diversity guided high-throughput approach to phage isolation and coverage optimization for an *E. coli* phage cocktail

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Bacteriophage based bacterial control strategies face two main challenges: (A) Within species diversity and (B) incapacity of many pathogens to drive their host to extinction. We address (A) by high-throughput phage isolation on maximally diverse target strains (>90) and a large environmental sample bank (225 manure and 225 sludge samples) and (B) by applying liquid isolation conditions which bypass plaque isolation and this way select the single most virulent phage from each environmental sample. By this approach we reached coverage saturation – i.e. the same coverage on the isolation target strains (94%) as on an *E. coli* collection of maximal diversity (ECOR collection) which is novel to the phages. By correlation analysis between infection patterns and presence/absence of virulence markers we could identify phages which preferentially infect *E. coli* with certain virulence surface markers (iron uptake, ompT, fimbriae etc.). Yet, no phage preferences were found for toxins, serotypes and antibiotic resistances. This demonstrates, that a simple correlation analysis can map *E. coli* diversity from a “phage eyes perspective”. Currently we use these strategies to optimize efficiency of a phage cocktail against Colibacillosis in weaning pigs.

(23) Canada Research Chair in Bacteriophages

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Using various examples, I will present the main goal of the Canada Research Chair in Bacteriophages, which is to improve knowledge of the biology of phages. We use an integrated approach that combines various classical virology assays and “omics” technologies to better understand interactions between phages and bacteria. New strategies are also developed to eliminate phages in food fermentations and to use phages as antibacterial agents in various industrial sectors and public health. The CRC in bacteriophages also manage the Félix d'Hérelle Reference Center for Bacterial Viruses, the largest collection of reference phages (www.phage.ulaval.ca). The missions of the Félix d'Hérelle Center are to collect, conserve and distribute reference phages and information about them to foster research and education.

(24) Diversité des bactériophages et rôles dans la fermentation malolactique des vins

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Oenococcus oeni assure la seconde fermentation du vin, appelée fermentation malolactique (FML). Cette étape est nécessaire à la vinification de presque tous les vins rouges et de nombreux vins blancs. Elle contribue au développement des caractéristiques organoleptiques du vin et participe à la stabilisation microbiologique du produit final. La FML est une étape souvent capricieuse, et les origines des arrêts ou non déclenchements de fermentation sont complexes. L'une des causes possibles est la destruction des souches bactériennes par des bactériophages spécifiques. Cette théorie est confortée par l'isolement fréquent de phages à partir de vins présentant des défauts de FML. Par ailleurs, une incidence de la lysogénie a été démontrée dans l'espèce, et les bactéries lysogènes seraient donc un réservoir potentiel de virus. Les objectifs de notre étude sont de caractériser la diversité des bactériophages infectant *O. oeni* et de comprendre l'impact des phages lytiques et tempérés dans le déroulement de la FML. Les résultats obtenus apporteront de nouvelles données sur la pertinence d'intégrer le critère de résistance aux phages dans le travail de sélection des levains FML commerciaux. Les travaux présentés sont réalisés dans le cadre de l'ANR LYSOPLUS.

(25) Specific targeting of O25b-ST131 *Escherichia coli* strains with an uncommon bacteriophage

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Amongst the highly diverse *E. coli* population, the O25b-ST131 clonal complex is of particular concern: as pathogenic strains they belong to the B2 phylogroup (where most extraintestinal-pathogenic *E. coli* classify), they express a large number of virulence factors and are involved in community as well as hospital-acquired infections. Since their first descriptions in limited countries in 2008, O25b-ST131 isolates are now present worldwide and associated with a high level of resistance to betalactams (mainly by producing CTX-M-15 extended spectrum betalactamase) and fluoroquinolones. Using an O25b-ST131 strain responsible for a nosocomial infection, we have isolated a bacteriophage, LM33-P1, that turns out to specifically infect O25b serotypes. From a collection (n=270) of *E. coli* strains displaying various O-type, we confirmed that LM33-P1 could only infect O25b strains. Within the 87 O25b strains

tested, 75% were lysed by this bacteriophage. We also showed that the specific interaction of LM33-P1 with O25b strains was LPS-dependent. Characterization of this bacteriophage revealed it was highly efficient in vitro (short eclipse and latent periods of respectively 7 and 9 minutes, burst size 320 pfu, fast adsorption) and also active in vivo, based on different animal models. Such bacteriophage is of particular interest for therapeutic approaches.

(26) Phage-Bacteria interactions in the gut: a story of therapy, ecology and evolution

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Both commensal and pathogenic *Escherichia coli* strains reside in the human gut and years of antibiotics pressure led to selecting for drug-resistant strains. Using mouse models, our team both studies the therapeutic potential of bacteriophages and the consequences of their use on evolution. Using mouse models, we showed effectiveness of bacteriophages cocktails in targeting both Uropathogenic *E. coli* (UPEC) and Adherent-Invasive *E. coli* (AIEC) associated to Inflammatory Bowel Disease (IBD) by significantly decreasing both intestinal colonisation and IBD symptoms of disease. A long-term gut co-colonisation model using one pathogenic and one commensal *E. coli* strain was setup to study adaptation of bacteriophage LF110_P1, which infects the pathogen but not the commensal. Remarkably, bacteriophages collected over time displayed adaptation to infect the commensal strain while keeping its infectivity towards the pathogen. In addition, such bacteriophage variants presented differential infectivity towards bacterial clones isolated from the gut. In parallel, experiments performed in vitro never led to obtaining such variants, suggesting a role for the gut environment in the adaptation mechanism. While the potential of phage therapy to target *E. coli* – associated gut infections is undisputable, the research into understanding the impact of bacteriophage-bacteria interactions on the gut ecology is still in its infancy.

POSTERS

(27) Pherecydes Pharma : lancement du premier essai clinique dans le monde, PHAGOBURN

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Pherecydes Pharma est le promoteur de la première étude mondiale multicentrique de phagothérapie chez le brûlé : PHAGOBURN. L'essai clinique de phase I/II est coordonné par l'hôpital d'instruction des armées Percy (SSA). Deux cocktails de bactériophages, mis au point par Pherecydes Pharma, ont été façonnés par le laboratoire Clean Cells et sont testés pour leur tolérance et leur efficacité chez les grands brûlés. Ils sont destinés à lutter contre les infections provoqués par *Escherichia coli* et , responsable d'infections nosocomiales pouvant entraîner la mort. Pherecydes Pharma met au point d'autres cocktails de bactériophages pour la lutte contre les infections respiratoires, les infections articulo-osseuse, ...

(28) Genetic Diversity of F-Specific RNA Bacteriophages isolated from Urban and Animal Waste as a Tool for Tracking the Origin of Fecal Pollution

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F-specific RNA bacteriophages (FRNAPH) are commonly used to evaluate and discriminate human from animal fecal/viral pollution. Indeed, while FRNAPH subgroup I (FRNAPH-I) and FRNAPH-IV are mostly detected in animal waste, FRNAPH-II and -III are frequent in human wastewater. Nevertheless, the lack of robustness of this repartition and the variable survival rates of subgroups in environment limit their use. In this study, we partially sequenced genomes of FRNAPH isolated from urban and animal feces/wastewater to increase their specificity. To limit the survival bias, the persistence of genomes and infectivity of each subgroup were studied in wastewater, over time and during treatment. FRNAPH-I were the most resistant and thus may be efficiently used as fecal/viral indicators but sequencing didn't allow to differentiate urban from animal strains. FRNAPH-II were uncommon in animal samples and low sequence variability associated with specific clusters formed by urban strains allowed to differentiate the origin of pollution by using a specific RT-PCR method. Their persistence was comparable to that of FRNAPH-I, but a higher elimination rates during activated-

sludge treatment was noted. Low sequence variability of animal FRNAPH-III strains also allowed the use of an urban specific RT-PCR method but low resistance restricted them to recent urban pollution detection.

(29) Bacteriophage loading of calcium phosphate bio-ceramics : a tool against infections in bone surgery

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Depuis plusieurs décennies, la composition minérale et la très bonne bio-activité des céramiques phosphocalciques (hydroxyapatite et phosphate tri-calcique) en font des matériaux de choix pour le remplacement des défauts osseux en chirurgie ostéo-articulaire. Suite à ces interventions chirurgicales, l'une des complications majeures demeure l'infection bactérienne dont le traitement par des antibiotiques est fréquemment inefficace en raison de leur faible diffusion jusqu'au site osseux. Ces dernières années ces infections nosocomiales ont été en constante augmentation de par le nombre de patients qui requièrent de telles interventions (vieillesse de la population) mais aussi de par l'augmentation de l'apparition des bactéries résistantes aux antibiotiques. Ce projet vise donc à pallier à ces deux problèmes, d'une part en travaillant sur la structure poreuse des céramiques combinées ou non avec des polymères organiques afin de pouvoir les charger de substances antibactériennes et assurer un relargage précis au cours du temps de ces antibactériens. D'autre part, l'utilisation d'antibiotiques ayant montré ses limites, l'utilisation d'un nouveau type de thérapie devient essentielle. La phagothérapie est l'une de ces thérapies. Cette thérapie présente l'avantage d'avoir une action ciblée rapide et très spécifique des souches bactériennes, évitant l'apparition d'effets secondaires et de réduire drastiquement l'apparition de nouvelles souches bactériennes résistantes, permettant un usage prophylactique assez aisé. Dans ce cadre, le LMCPA a démontré la capacité naturelle des céramiques phosphocalciques de retenir et relarguer les bactériophages jusqu'à 6 jours, les capacités de rétention et les cinétiques de relargage étant plus ou moins bonnes en fonction des taux de microporosité des céramiques. A terme, il s'agit de développer un dispositif de phagothérapie localisée, préventif et curatif en chirurgie ostéo-articulaire.

(30) Factors affecting the effectiveness of phage use against pathogenic bacteria

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In recent years, the use of lytic bacteriophages as antimicrobial agents controlling

pathogenic bacteria has appeared as a promising new alternative strategy in the face of growing antibiotic resistance which has caused problems in many fields including medicine, veterinary medicine and aquaculture. The use of bacteriophages has numerous advantages over traditional antimicrobials. The effectiveness of phage applications in fighting against pathogenic bacteria depends on several factors such as the bacteriophages/target bacteria ratio, the mode and moment of treatment, environmental conditions (pH, temperature ...), the neutralization of phage and accessibility to target bacteria, amongst others. This poster presents these factors involved in developing phage therapy applications.

(31) Counting and sorting biotic nanoparticles from aquatic environments by full field interferometry

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We developed a new common path interferometric light microscope that takes advantage of the interference between a strong optical reference signal and the weak power scattered by the nano-object or biotic nanoparticles such as viruses or vesicles to enhance the detected signal. The nanoparticles are localized close to the focal plane of a large numerical aperture microscope objective. The particle, much smaller than the wavelength, radiates a spherical wave whose center is located close to the focus of the microscope objective. This scattered wave interferes with the incoming beam constructively or destructively when the particle is located before or after the objective focus, respectively. The corresponding diffraction spot, recorded by the pixels of the camera is slightly larger or smaller than the background reference level. Our method couples two measurements: detected signal amplitude and Brownian motion jumps. Rayleigh scattering amplitude is dependent on the size of the spherical particles; it is proportional to the third power of their diameter and to their refractive index difference Δn to water refractive index. Thus, from the maximum intensity distribution and the jumps between successive images of any given tracked particle, we can deduce its diameter by the two different approaches.

(32) Bacterial vaginosis: Are bacteriophages involved in dysbiosis of human genital tract?

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Throughout the world, Bacterial Vaginosis (BV) is the most prevalent form of vaginal infection in women of reproductive age. This dysbiosis increases risks of reproductive complications, preterm delivery and susceptibility to sexually transmitted infections. Within a genital tract, BV is characterized by: (i) the depletion of most resident commensal *Lactobacillus* spp.; (ii) an increase in bacterial diversity; and (iii) the overgrowth of pathogenic anaerobic bacteria probably owing to growth of *Gardnerella vaginalis* and establishment of biofilms. Unfortunately, the etiology of BV is still unclear. Moreover, BV is a synergistic polymicrobial syndrome for which antibiotic therapies currently fail in the long-term. Phage therapy may thus be an alternative for treatment. The aim of our project is to determine and further test experimentally how bacteriophages (both lytic and temperate) are involved in the etiology of BV. To achieve our objective, we first tested for the presence of temperate bacteriophages isolated and expressed (with mitomycin C) from *Lactobacillus* spp. sampled from healthy and dysbiotic genital tracts. Out of 32 isolated lactobacilli, eleven expressed prophages had head-tail structures characteristic to Siphoviridae, and 17 had isometric capsids either without tails or with very short tails. To determine the identity and polymorphism of phages, we plan to sequence their complete genomes using the Illumina HiSeq2500 platform with multiplexing. We further plan to test their infectivity and host-range.

(33) Selective pressure imposed on *Pseudomonas aeruginosa* by bacteriophages: the importance of pseudolysogeny

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Bacteriophages have been shown to drive the emergence of *P. aeruginosa* variants playing a significant role in bacterial survival, activity, and evolution. As therapeutic agents, they can be employed to defeat pathogens difficult to eradicate with common antimicrobial agents, although, as for antibiotics, they can select phage-resistant variants. The purpose of this study was to investigate the effect of development of phage-resistance on selected phenotypic traits and virulence determinants of *P. aeruginosa*. Phage-resistant variants with alterations in bacterial motility and biofilm formation, or with a mucoid phenotype were specifically selected according to the phage used for the infection both alone or in a cocktail. The genome of several variants was sequenced, and mutations in genes involved in the biogenesis of the type IV pilus, LPS production and in the alginate regulation were identified. Some variants which genome was not mutated, were shown to contain phage DNA suggesting that immunity was driving resistance to phages. Alterations in biofilm formation and bacterial motility, as well as mucoidy are factors that can highly influence the virulence potential of phage-resistant variants. It is therefore essential to further investigate bacterial resistance to phage to anticipate the potential effects of

phage therapy.

(34) PHAGOBURN: Back to the Phuture !

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The European clinical trial PHAGOBURN has been initiated on July 22 by including the first patient. The process to reach that important milestone during two years of extensive efforts includes the setup of a manufacturing capacity to produce two clinical batches at Clean Cells Co. with the support of Pherecydes Pharma Co. the sponsor. Both tested phage cocktails were developed by the sponsor: the first one PP0121 is dedicated to fight *E. coli* infections, whereas the second (PP1131) one targets *P. aeruginosa*. The PHAGOBURN is a Phase I/II randomized clinical trial in the area of human phage therapy to assess the efficacy and safety of each product in comparison with a control group (silver sulfadiazine). This is a multi-centric study involving 11 different burn units located in France, Belgium and Switzerland. This is a four arms assessor blind study. The primary endpoint of the trial is the time for reduction of the targeted bacterial load in wound burns with a specific designed microbiological procedure. In addition, the bacterial response to individual phage active substances from each product and to the whole PP0121 and PP1131 cocktails is being evaluated.

(35) A survey of oenophages during winemaking reveals a novel group with unusual genomic characteristics

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During winemaking the predation of *O. oeni* by bacteriophages, called oenophages, has often been demonstrated. However our current knowledge of oenophage diversity is still limited. In a previous study, we proposed a classification scheme for oenococcal prophages based on integrase gene polymorphism. Prophagic sequences were observed to be clustered into four integrase groups. PCR targeting the integrase sequence was subsequently used to analyze different phages isolated from red wine samples collected in the Pauillac area (France). Accordingly, a distribution into four groups was observed. The objective of the current study was to analyze a broader collection of samples from different grape varieties and vineyards.

A total of 166 samples were collected during the 2013 and 2014 vintages. Presence of active phages was assessed not only in wines during MLF, but along the whole vinification process. Samples were therefore taken from must, during alcoholic (AF) or malolactic fermentation (MLF) and ageing. Our survey demonstrated that phages are mostly found in must samples, at the end of the AF and during MLF. PCR typing revealed a novel group of oenophages with unusual genomic characteristics. Oenophages are therefore more diverse than expected and our work provides new insight into phage diversity, offering new tools to monitor and assess the ecological significance of bacteriophages in the oenological environment.

(36) Immunocompatibility of Respiratory Phage Therapy

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The use of antibiotics is unavoidable in treating respiratory tract infections in patients with cystic fibrosis (CF). However, physicians are increasingly faced with multidrug-resistant *Pseudomonas aeruginosa*. Phages have shown preclinical efficacy in treating respiratory tract infections caused by *P. aeruginosa* strain PAK in healthy subjects. This study aimed at evaluating if the same phage treatment is effective in adaptive (Rag2-/- γ c-) and innate (Myd88-) immunodeficient mice. We demonstrate that the pseudomonal phage PAK_P1, intranasally administered 4d before or 2h after strain PAK intranasal challenge, respectively prevented or cured 100% of respiratory infections in Rag2-/- γ c- mice. Mice lacking MyD88, a key player in the innate immune system, were 100-fold more sensitive to strain PAK infection. As a result, phage PAK_P1 treatment did not prevent or cure *P. aeruginosa* infections in Myd88- mice, even after the bacterial inoculum was reduced 100-fold. In addition, 24h after intranasal administration of phage PAK_P1 in non-challenged WT mice, lung homogenates did not show any induction of 20 cytokines and chemokines surveyed. In conclusion, phage PAK_P1 therapeutic ability was dependent on contribution from the subjects' innate immune system, but not the adaptive immune system. Furthermore, the phage particles themselves do not illicit a proinflammatory response, suggesting phage PAK_P1 is nontoxic.

(37) Characterization of lytic bacteriophages from *Leptospira* spp.

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Leptospirosis is a neglected zoonotic disease caused by *Leptospira interrogans*. Leptospirosis has a global distribution, and is considered as an emerging infectious disease. To improve our knowledge on ecology of leptospire, we are looking for *Leptospira* spp. bacteriophages. "Leptophages" could be a potential source of genetic

tools, and their bactericidal activity may be used for veterinary treatments. This work presents optimization of leptophage production and visualization by Transmission Electron Microscopy (TEM).

(38) Caractérisation de bactériophages actifs contre des colibacilles pathogènes aviaires

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Chez la volaille, *Escherichia coli* est l'agent étiologique de la colibacillose qui est une pathologie majeure de la filière avicole. Ces dernières années, on assiste à une augmentation inquiétante du nombre de cas tant dans les élevages de poules pondeuses, chez certaines souches de reproducteurs chair et chez les poulets de chair à croissance rapide. Le traitement de la colibacillose reposant essentiellement sur l'utilisation de l'antibiothérapie, le nombre de souches résistantes aux antibiotiques augmente, telle que la résistance aux céphalosporines de 3^{ème} et 4^{ème} génération (12,2% de souches résistantes en 2009, données Resapath). Dans l'objectif de maîtriser la colibacillose aviaire par la phagothérapie et dans le cadre du projet européen « Antibioophage » (2015-2019, call Anihwa) nous avons dans un premier temps cherchés des coliphages à partir de différents types d'échantillons (eaux, contenu caecal d'espèces variées d'oiseaux). Nous avons ainsi isolés 5 coliphages différents pour lesquels le spectre d'hôte, la morphologie en microscopie électronique et la séquence génomique ont été déterminés. Ces coliphages sont apparentés à des phages récemment décrits. Leur efficacité à provoquer la clairance bactérienne sera évaluée *in vitro* et *in vivo*.

(39) Du phage T4 aux herpes virus, peut on parler du plus vieux modèle de co-evolution ?

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Les herpes virus (HV) sont des virus à ADN double brin présents des mollusques (Mollusca herpesviridae) à l'homme (Herpesviridae sensu stricto), soit à l'état épisomique, comme dans le cas du VZV chez l'homme, ou encore d'OSHV1, le virus impliqué dans les mortalités estivales de l'huître creuse *Crassostrea gigas*; soit encore intégré au génome de l'hôte (comme dans le cas de l'herpès de l'abalone...). Le comportement des HV ressemble de fait à celui de phages lysogènes chez la bactérie dont le modèle d'étude est le phage T4. Des études structurales de la

seringue à ADN phagique ont d'ailleurs montré des similarités de structure avec son homologue fonctionnel chez les herpes. Dans la mesure où les outils de la phylogénie classique sont inaptes à retracer des histoires évolutives sur de très grandes périodes nous avons tenté d'évaluer le concept de synténie positionnelle pour transposer l'annotation génomique des herpes humains sur les génomes de malacoherpeviridae et au delà.