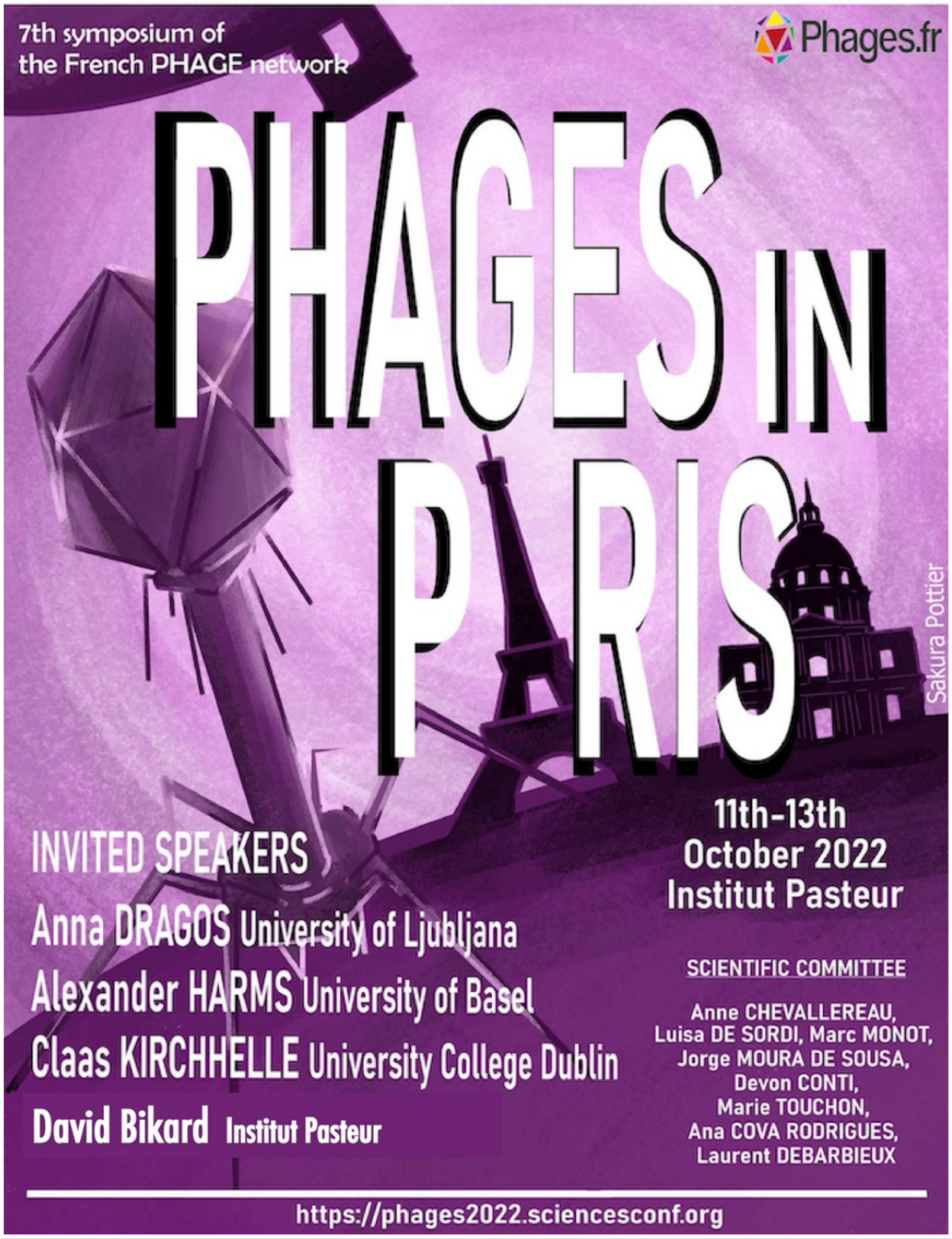
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Planning

Tuesday, October 11, 2022

TIME	EVENT
13:00 - 14:00	Registration
14:00 - 14:15	Welcome Talk
14:15 - 15:55	**Phage-Host Interactions Session**
14:15 - 14:55	› No need to catch 'em all: Exploring new worlds of phage-host interactions with handpicked natural isolates - *Alexander Harms, Biozentrum, University of Basel*
14:55 - 15:15	› Phage-plasmids are prevalent and produce antibiotic resistant lysogens - *Pfeifer Eugen, Microbial Evolutionary Genomics, Department Genomes and Genetics*
15:15 - 15:35	› Phage-inducible chromosomal islands promote genetic variability by blocking phage reproduction - *Rodrigo Ibarra-Chavez, Institute of Infection, Immunity & Inflammation - Biomedical Research Center, University of Copenhagen*
15:35 - 15:55	› A new phage satellite discovered in natural population of vibrio - *Frédérique LE ROUX, Ifremer Unité Physiologie Fonctionnelle des Organismes Marins*
15:55 - 16:30	Coffee break
16:30 - 17:50	**Phage-Host Interactions Session**
16:30 - 16:50	› A novel RmuC domain-containing protein mediates phage defence in Pseudomonas spp. - *Giuseppina Mariano, Biosciences Institute, Cookson Building, Faculty of Medical Sciences, Newcastle University, Framlington Place Newcastle upon Tyne NE2 4HH*
16:50 - 17:10	› ATP nucleosidases are conserved immune effectors in Prokaryotes and Eukaryotes - *Francois Rousset, Department of Molecular Genetics, Weizmann Institute of Science*
17:10 - 17:30	› Translation inhibitor antibiotics decrease immunosuppression induced by anti-CRISPR proteins - *Benoit PONS, College of Life and Environmental Sciences [Exeter], University of Exeter*
17:30 - 17:50	› DNA motif-based Discovery of Anti-defense Genes in Archaea viruses - *Yuvaraj Bhoobalan, University of Copenhagen,*

Wednesday, October 12, 2022

TIME	EVENT
08:30 - 10:10	**Ecology and Evolution Session**
08:30 - 09:10	› Phages and their satellites encode hotspots of antiviral systems - *David Bikard, Institut Pasteur*
09:10 - 09:30	› Découverte de virus fusiformes infectant des archées méthanogènes, par le couplage de marquage isotopique (stable isotope probing) et de métagénomique - *Ariane Bize, Université Paris-Saclay, INRAE, PROSE*
09:30 - 09:50	› Analysing active interactions between autotrophs and viruses in soil by following transfer of assimilated carbon - *Sungeun LEE, Laboratoire Ampère*

TIME	EVENT
09:50 - 10:10	› Compartimentation différente de l'antibiorésistance entre phages et bactéries dans un continuum rivière - effluents de station d'épuration. - *Charlène SAGRILLO, Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement*
10:10 - 10:45	Coffee break
10:45 - 12:05	**Ecology and Evolution Session**
10:45 - 11:05	› The role of capsules and depolymerases in Klebsiella-phage interactions - *Beatriz Beamud, Synthetic Biology, Institut Pasteur*
11:05 - 11:25	› Competition between phage-resistance mechanisms determines the outcome of bacterial co-existence - *Olaya Rendueles, Microbial Evolutionary Genomics, Institut Pasteur, Paris*
11:25 - 11:45	› Demographic coexistence or coevolution ? Epidemiological dynamics of E.Coli during infection by bacteriophages T7 - *Yoann Anciaux, Institut des Sciences de lÉvolution de Montpellier, UMR 5290 MIVEGEC*
11:45 - 12:05	› Phage-assisted directed evolution of proteins and RNAs - *Alfonso Jaramillo, i2SysBio, CSIC-University of Valencia, Paterna*
12:05 - 12:15	Flash talks
12:15 - 14:15	Lunch/Posters
14:15 - 15:55	**Phage-Host Interactions Session**
14:15 - 14:55	› Control of spore-forming bacteria by phage regulatory switches - *Anna Dragoš, Biotechnical Faculty, University of Ljubljana*
14:55 - 15:15	› Prophage taming by the adherent-invasive Escherichia coli LF82 upon macrophage infection - *Pauline Misson - MICALIS*
15:15 - 15:35	› YbcM, a transcriptional regulator from prophage origin involved in Escherichia coli physiology - *Nolan Tronche, Laboratoire de chimie bactérienne, UMR7283, Institut de Microbiologie de la Méditerranée*
15:35 - 15:55	› Choreography of phages PAK-P3 and PhiKZ genomes during Pseudomonas aeruginosa infection. - *Amaury BIGNAUD, Regulation Spatiale des Genomes, Institut Pasteur*
15:55 - 16:30	Coffee break
16:30 - 17:50	**Phage-Host Interactions Session**
16:30 - 16:50	› Analysis of the microbiome and virome of a gnotobiotic mouse model by Chromosome Conformation Capture - *Quentin Lamy-Besnier, Regulation Spatiale des Genomes, Laboratoire Bactériophage, Bactérie, Hôte*
16:50 - 17:10	› Membrane-less compartimentalization in time and space of bacteriophage SPP1 replication and assembly in the Gram-positive bacterium Bacillus subtilis. - *Audrey Labarde, Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France*
17:10 - 17:30	› Structural basis of cell wall perforation by bacteriophage T5 - *Cécile Breyton, Institut de Biologie Structurale*
17:30 - 17:50	› Raising the bar in undergraduate teaching: discovery and characterization of phages infecting Corynebacterium glutamicum by Bachelor students at Paris-Saclay University - *Christophe*

TIME	EVENT
	Regeard, Institute for Integrative Biology of the Cell - Ombeline ROSSIER, Institut de Biologie Intégrative de la Cellule
17:50 - 18:00	Flash talks
18:00 - 20:30	Posters/Conference Dinner

Thursday, October 13, 2022

TIME	EVENT
08:30 - 10:10	**Therapy and Biotechnology Application Session**
08:30 - 09:10	› Beyond boom and bust: re-evaluating the history of West-European phage therapy - *Claas Kirchhelle, University College Dublin*
09:10 - 09:30	› PHAGEinLYON / PHAG-ONE : Optimization of pharmaceutical production of anti-S. aureus therapeutic phages using in silico and experimental approaches - *Mathieu Medina, Institut des Agents Infectieux, Laboratoire de Bactériologie, Equipe Pathogénie des Infections à Staphylocoques*
09:30 - 09:50	› Directed in vitro evolution of virulent bacteriophages against Enterococcus faecium following the Appelmans Protocol - *Julien Lossouarn, Institut Micalis*
09:50 - 10:10	› Evolutionary training: how bacteriophages adapt to multiple strains of Salmonella enterica - *Amandine Maurin, CNRS, UMR 224 MIVEGEC (IRD, UM, CNRS), Montpellier*
10:10 - 10:45	Coffee break
10:45 - 12:05	**Therapy and Biotechnology Application Session**
10:45 - 11:05	› Evaluation of the prophylactic and therapeutic effect of a phage cocktail to control Salmonella Enteritidis in poultry - *Lorna AGAPE, INRAE*
11:05 - 11:25	› Adaptation of the Group A Streptococcus bacteriophages to the human host - *Lionel Schiavolin, Molecular Bacteriology Laboratory, Université Libre de Bruxelles*
11:25 - 11:45	› Investigations of the role of neutrophils and macrophages in immunophage synergy during experimental pulmonary phage therapy - *Sophia Zborowsky, Institut Pasteur, Université Paris Cité, CNRS UMR6047, Bacteriophage Bacterium Host, Paris 75015, France*
11:45 - 12:05	› Face à l'antibiorésistance, une écologie politique des microbes - *Charlotte Brives, Centre Émile Durkheim, Université de Bordeaux, Sciences Po, CNRS : UMR5116*
12:05 - 12:15	Meeting conclusions
12:15 - 13:15	AG Phages.fr

Oral Presentations

Session 1 – Phage-host interactions

Session 2 – Ecology and Evolution

Session 3 – Phage-host interactions

Session 4 – Applications in Therapy and Biotechnology

[Keynote] No need to catch ‘em all: Exploring new worlds of phage-host interactions with handpicked natural isolates

Alexander Harms[1]

1 : Biozentrum, University of Basel

Research on the biology of bacteriophages and their application in clinics and biotechnology is currently booming. However, most work on the molecular mechanisms of phage-host interactions has focused on few model phages, suggesting that natural phage diversity holds a huge untapped potential. We therefore composed a “BASEL collection” of around 80 new isolates that cover all major groups of tailed phages infecting *Escherichia coli*. This collection can serve as a powerful tool to probe a meaningful proportion of phage diversity when exploring any aspect of phage-host interactions, and we freely share these phages with researchers around the world. Our own genomic and phenotypic analyses directly resulted in remarkable discoveries, e.g., regarding the molecular basis of phage receptor specificity or the sensitivity / resistance of different phage groups to bacterial immunity systems.

In parallel, we studied how bacteriophages infect slow- or non-growing bacteria that dominate in most ecosystems and are a major cause of chronic or relapsing infections due to their notorious antibiotic tolerance. Intriguingly, most phages failed to productively infect dormant hosts and instead entered an enigmatic state of hibernation previously described as “pseudolysogeny”. However, we isolated a new phage called Paride that uniquely wipes out deep-dormant cultures of *Pseudomonas aeruginosa* irrespective of their extreme antibiotic tolerance. We are currently studying the molecular mechanisms underlying this ability. This work will shed new light on the biology of phages in natural environments and might identify Achilles' heels in the resilient physiology of dormant bacteria that could be targeted by new antimicrobials.

Phage-plasmids are prevalent and produce antibiotic resistant lysogens

Pfeifer Eugen[1] , Jorge Sousa[1], Marie Touchon[1], Rémy Bonnin[2], Eduardo Rocha[1]

1 : Microbial Evolutionary Genomics, Department Genomes and Genetics
Institut Pasteur de Paris, CNRS : UMR3525
2 : Team "Resist" UMR1184 "Immunology of Viral, Auto-Immune, Hematological and Bacterial diseases (IMVA-HB),"
Institut National de la Santé et de la Recherche Médicale - INSERM, Université Paris-Saclay,Sorbonne Universités : CEA, LabEx LERMIT, Faculty of Medicine, Le Kremlin-Bicêtre, France, French National Reference Center for Antibiotic Resistance: Carbapenemase producing Enterobacteriaceae [Le Kremlin-Bicêtre]

Plasmids are DNA molecules that replicate autonomously within cells and are usually thought to transfer by conjugation. In the literature they are very clearly distinguished from prophages of temperate phages, which are usually described as integrating the host chromosome. Some elements, termed phage-plasmids, blurry these definitions by infecting bacteria as phages and replicating as plasmids. However, their number, diversity, and contribution to bacterial phenotypes remained poorly known.

To study phage-plasmids, we first developed a screen to detect them in bacterial genomes, plasmid and phage databases. Strikingly, we found phage-plasmids to be numerous and prevalent (5% of >25k phages/plasmids) in Prokaryotes. We computed and used the gene repertoire relatedness to classed them into different groups. Interestingly, most groups are not related, suggesting independent origins. Nevertheless, all elements have typical phage and plasmid features, suggesting that phage-plasmids are diverse and ancient [1]. Phages rarely encode antibiotic resistance genes (ARGs) whereas plasmids often do. What about phage-plasmids which have mixed features of both types of elements? We find that ARGs are 80 times more frequent in phage-plasmids than in phages and 5 times less than in plasmids. Phage-plasmids transfer as phages. Could they spread ARGs as viruses? To test this, we isolated phage-plasmids from Carbapenem-resistant enterobacterial strains that were retrieved from clinical samples and conducted induction and infections experiments. We confirmed that phage-plasmids of different types, and encoding different ARG classes, are inducible by mitomycin C and generate antibiotic resistant lysogens after infection [2].

In conclusion, our results show that phage-plasmids are abundant elements are capable of transferring accessory genes typical of phages and of plasmids. This point out, that they may have a remarkable impact on bacterial evolution.

[1] E Pfeifer, JA de Sousa, M Touchon, EPC Rocha, 2021, NAR, doi: 10.1093/nar/gkab064

[2] E Pfeifer, R Bonnin, EPC Rocha, 2022, bioRXiv, https://doi.org/10.1101/2022.06.24.497495

Keywords: PhagePlasmids; AMR genes; comparative phage genomics

Phage-inducible chromosomal islands promote genetic variability by blocking phage reproduction

Rodrigo Ibarra-Chavez[1,2], Aisling Brady[3,2], John Chen[4] , José Penadés[3,2], Andreas Haag[5,2]

1 : University of Copenhagen
2 : Institute of Infection, Immunity & Inflammation - Biomedical Research Center
3 : MRC Centre for Molecular Microbiology and Infection [Imperial College, London]
4 : National University of Singapore
5 : School of Medicine [University of st Andrews]

Phage-inducible chromosomal islands (PICIs) are a widespread family of highly mobile genetic elements that disseminate virulence and toxin genes among bacterial populations. Since their life cycle involves induction by helper phages, they are important players in phage evolution and ecology. PICIs can interfere with the lifecycle of their helper phages at different stages resulting frequently in reduced phage production after infection of a PICI-containing strain. Since phage defense systems have been recently shown to be beneficial for the acquisition of exogenous DNA via horizontal gene transfer, we hypothesized that PICIs could provide a similar benefit to their hosts and tested the impact of PICIs in recipient strains on host cell viability, phage propagation and transfer of genetic material. Here we report an important role for PICIs in bacterial evolution by promoting the survival of phage-mediated transductants of chromosomal or plasmid DNA. The presence of PICIs generates favorable conditions for population diversification and the inheritance of genetic material being transferred, such as antibiotic resistance and virulence genes. Our results show that by interfering with phage reproduction, PICIs can protect the bacterial population from phage attack, increasing the overall survival of the bacterial population as well as the transduced cells. Moreover, our results also demonstrate that PICIs reduce the frequency of lysogenization after temperate phage infection, creating a more genetically diverse bacterial population with increased bet-hedging opportunities to adapt to new niches. In summary, our results identify a new role for the PICIs and highlight them as important drivers of bacterial evolution.

Keywords: Bacteriophages; Phage satellites; Genetic interference; Bacterial evolution

A new phage satellite discovered in natural population of vibrio

Frédérique Le Roux[1] , Rubén Barcia-Cruz, Damien Piel, David Goudenege, Jorge Sousa, Martial Marbouty, Eduardo Rocha

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In the marine bacteria from the *Vibrionaceae* family, many genomic islands are selected for and transferred by unknown mechanisms. We recently showed that numerous phage defense systems are encoded on genomic islands (Piel et al, Nat Micro 2022) but the mechanisms of transfer of these elements remained to be explored. Phage satellites are mobile genetic elements that exploit phages to ensure their own propagation and horizontal transfer into new bacterial hosts. We found that phage satellites are extremely abundant and broadly distributed in the vibrios and we discovered a new satellite family. Compared to known satellites, these elements are smaller in size, carried only 6 to 12 genes and are always integrated in the same recombination site (end of the Fis regulator gene). The activation of the element (excision and replication) is induced by specific virulent phage (or helper phage) and requires 4 genes encoding a putative excisionase (*alpA*), a tyrosine recombinase (*int*), a primase and a protein of unknown function, the latter being satellite-specific. Once excised and replicated, the element hitchhikes the capsid of the helper phage and can be transduced in a new host. We showed that this element is involved in the defense against other specific phages. We speculate that this "exclusion system" guarantees the helper phage a population of hosts to replicate.

Keywords: Mobile genetic element; Phage defence; Natural population

A novel RmuC domain-containing protein mediates phage defence in Pseudomonas spp.

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Competitive bacteria-bacteriophage interaction has resulted in the evolution of numerous bacterial defence systems that prevent phage propagation, with phage co-evolving counter-resistant strategies, resulting in a stable equilibrium in the natural environment.

In recent years, computational and bioinformatic studies have allowed tremendous advances in discovering novel bacterial defence systems, rendering increasing clear that many more systems still await discovery. These systems are encoded within defence islands, genomic loci where many distinct defence systems are clustered together.

Here we report the identification of a novel antiphage system that is part of *Pseudomonas* defence arsenal. We identify six subtypes of the novel system, which share a core component that harbours a RmuC domain. Through phenotypic and biochemical characterisation of its components, we demonstrate that this system provides protection against a panel of different phages and that the RmuC domain is crucial for defence. The novel systems were renamed ShieldI-VI, and their RmuC-harbouring core component was renamed ShdA. We show that ShdA can degrade phage DNA *in vitro* and alter the normal nucleoid organisation of chromosomal DNA. Collectively, our data identify a new player within *Pseudomonas* bacterial immunity arsenal that displays a novel mode of action.

Keywords: Bacterial immunity; DNAse; Phage defence

ATP nucleosidases are conserved immune effectors in Prokaryotes and Eukaryotes

Francois Rousset[1]

1 : Department of Molecular Genetics, Weizmann Institute of Science

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The billion-year-old coevolution between bacteria and phages has led to the emergence of an arsenal of defense strategies to overcome phage infection. Among them, the CBASS family of anti-phage systems involves the production of cyclic nucleotide messengers upon infection, which bind and activate CBASS immune effectors. While several CBASS effectors have been characterized, such as nucleases and phospholipases, the molecular function of others remains unknown. Here, we report that some CBASS effectors act as ATP nucleosidases, breaking down ATP molecules into adenine and ribose-triphosphate during phage infection. Using a phylogenetic approach, we found that ATP nucleosidases are mobilized by additional defense systems, including a novel one we term Detocs. Finally, we show that this defense strategy is also manifested in the innate immune system of Eukaryotes. Taken together, our findings establish that ATP nucleosidases are immune effectors across the tree of life.

Keywords: immunity; CBASS; anti-phage defense

Translation inhibitor antibiotics decrease immunosuppression induced by anti-CRISPR proteins

Benoit Pons[1,2], Tatiana Dimitriu[1,2], Edze Westra[1,2], Stineke Van Houte[1,2]

1 : College of Life and Environmental Sciences [Exeter]
2 : University of Exeter

Pathogenic bacterial infections can be treated by phage therapy, sometimes in combination with antibiotics. However, bacteria have a wide range of defence mechanisms against phage, among which is CRISPR-Cas. CRISPR-Cas system relies on storage of phage genetic material from previous failed infection which is then used to guide sequence-specific cleavage of phage genetic material in subsequent infections. On the other hand, phages have evolved counter-defence mechanisms, such as anti-CRISPR (Acr) proteins. These proteins are expressed in the early stages of the infection and can inhibit one or several steps of the CRISPR-Cas mechanisms to allow the phage to replicate.

Since Acr-phage infection success depends on a strong and early expression of Acr proteins, we hypothesized that translation inhibitor antibiotics could disadvantage Acr-positive phages when infecting CRISPR immune bacteria. Consistently with this hypothesis, we show that sub-inhibitory doses of translation inhibitor antibiotics decrease the efficiency of AcrIF1 from phage DMS3vir in its host *Pseudomonas aeruginosa* PA14 carrying a type I-F CRISPR-Cas system. As a result, when infecting CRISPR immune cells by Acr-phage, the presence of antibiotics prevents phage amplification while protecting bacteria from lysis. These results highlight a potential role for translation inhibitors when selecting antibiotics for combined phage-antibiotics therapy.

Keywords: CRISPR-Cas; Anti-CRISPR; translation inhibitors

DNA motif-based Discovery of Anti-defense Genes in Archaea viruses

Yuvaraj Bhoobalan[1], Shuanshuan Xu[2], Laura Martinez-Alvarez[2], Xu Peng[2]

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In silico identification of novel anti-CRISPRs (Acrs) has relied completely on the guilt-by-association method with Acrs or anti-CRISPR associated proteins (Acas) as bait. Limited distribution of the characterized archaeal Acrs and Aca hinders our ability towards identifying new Acrs using the aforementioned method. In this paper, we leverage the possibility that all anti-defence genes ought to be expressed immediately post infection and hence must carry optimal and identical promoters, to identify anti-defense elements (ADE) within viruses without any prior knowledge of their protein functions. Using this consensus sequence based method, we have identified several potential anti-defense genes within archaeal viruses. Experimental screening identified the first subtype I-A inhibitor as well as the first virally encoded inhibitor of an archaeal abortive infection system within lytic and temperate viruses of archaea. Furthermore, we have also identified regulatory proteins potentially akin to Acas, which can lead to further identification of anti-defense genes in combination with the neighborhood method. These results demonstrate an approach for the extensive identification of ADEs among archaeal viruses with potential to be relevant among bacteriophages.

Keywords: phage anti-defence mechanisms; Anti-CRISPR

[Keynote] Phages and their satellites encode hotspots of antiviral systems

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Bacteria carry diverse genetic systems to defend against viral infection, some of which are found within prophages where they inhibit competing viruses. Phage satellites pose additional pressures on phages by hijacking key viral elements to their own benefit. Here, we show that E. coli P2-like phages and their parasitic P4-like satellites carry hotspots of genetic variation containing reservoirs of anti-phage systems. We validate the activity of diverse systems and describe PARIS, an abortive infection system triggered by a phage-encoded anti-restriction protein. Antiviral hotspots participate in inter-viral competition and shape dynamics between the bacterial host, P2-like phages, and P4-like satellites. Notably, the anti-phage activity of satellites can benefit the helper phage during competition with virulent phages, turning a parasitic relationship into a mutualistic one. Anti-phage hotspots are present across distant species and constitute a substantial source of systems that participate in the competition between mobile genetic elements.

Découverte de virus fusiformes infectant des archées méthanogènes, par le couplage de marquage isotopique (stable isotope probing) et de métagénomique

Hoang Vuong Quoc Hoang[1], François Enault[2], Cédric Midoux[3], Mahendra Mariadassou[4], Olivier Chapleur[1], Laurent Mazéas[1], Valentin Loux[5], Théodore Bouchez[1], Mart Krupovic[6], Ariane Bize[1]

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La diversité des virus infectant les archées non-extrémophiles a été grossièrement sous étudiée. C'est en particulier le cas pour les virus infectant les archées méthanogènes, qui sont pourtant des acteurs clés dans le cycle biogéochimique global du carbone. Une dizaine seulement de tels virus ont été isolés jusqu'à présent. Dans notre étude, nous avons mis en œuvre un couplage original entre le marquage isotopique (*Stable Isotope Probing*, SIP), et des analyses complémentaires de métagénomique *shotgun*, pour identifier des virus infectant des archées méthanogènes impliquées dans la bioconversion du formate au sein d'écosystèmes de digestion anaérobie. Pour cela, nous avons employé ce substrat comme seule source de carbone au sein de microcosmes de méthanisation.

Les analyses métagénomiques, incluant l'analyse des contigs viraux ainsi que la prédiction de leur hôte en utilisant des bases de données de *spacers*, ont abouti à la découverte de plusieurs génomes viraux qui n'étaient pas connus jusqu'à présent. En particulier, nous avons obtenu le génome complet d'un caudovirus infectant *Methanobacterium*, et qui représente une nouvelle famille virale (*Speroviridae*). Nous avons également identifié des génomes quasi-complets de virus fusiformes, dont les hôtes prédits appartenaient respectivement aux genres *Methanobacterium* et *Methanosarcina*. Ils font partie des premiers virus de morphotype fusiforme identifiés pour les archées méthanogènes.

De plus, les virus d'archées déjà connus et l'ensemble des contigs viraux susceptibles d'être associés à des archées méthanogènes ont été analysés par des réseaux de gènes-partagés. Ceci a mis en évidence la dominance de caudovirus, néanmoins relativement éloignés de ceux déjà connus, et la présence de virus différents, sans doute spécifiques aux archées, tels que les deux virus fusiformes évoqués ci-dessus.

Ainsi, notre approche expérimentale originale permet d'identifier les virus infectant des groupes fonctionnels clés, contribuant aux flux biogéochimiques au sein de communautés de microbes non-cultivés. Elle pourrait être employée pour étudier les virus infectant les microorganismes actifs dans tout type d'écosystème. De plus, elle pourrait être développée en appliquant le SIP directement aux ADN viraux, et nous en avons établi la preuve de concept en utilisant le modèle biologique *Escherichia coli* et son bacteriophage T4.

Keywords: diversité virale; virus d'archées; métagénomique; stable isotope probing ; prédiction d'hôtes ; digestion anaérobie

Analysing active interactions between autotrophs and viruses in soil by following transfer of assimilated carbon

Sungeun Lee[1], Christina Hazard[1], Graeme Nicol[1],

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Ecole Centrale de Lyon

While we have substantial knowledge of the complexity of prokaryotic communities and their contribution to functional processes in soil, we are currently ignorant on the role of viruses in influencing their ecology. In particular, difficulties remain in identifying the frequency of active interactions between host and virus populations in situ, largely due to a lack of tools to study interactions within this highly diverse and heterogeneous environment. To examine discrete, active interactions between individual host and virus populations in situ, this study focused on the transfer of assimilated carbon from autotrophic hosts to viruses. Microcosms containing pH 4.5 or 7.5 soil were amended with 13C methane or carbon dioxide and subjected to short-term incubations. Using DNA stable-isotope probing combined with metagenomic analyses, microbial networks were characterised, with primary and secondary utilisers of carbon identified, together with the transfer of host-fixed carbon to viruses. In methane incubated soils, 63% of 13C-enriched viral contigs were associated with methylotrophic bacteria with viruses non-methanotrophic methylotrophs and predator bacteria also identified. Active interactions and history of virus interactions with individual hosts were also characterised via analysis of 13C-enriched virus protospacer sequences in host CRISPR arrays. In soils incubated with carbon dioxide under nitrifying conditions, active viruses of ammonia oxidising archaea and nitrite oxidising bacteria were identified. Putative viruses of both methane oxidisers and ammonia oxidising archaea contained auxiliary metabolic genes involved in central metabolic pathways, and analysis of viral genes (e.g. integrases, tail and capsid proteins) revealed that they were distinct from previously cultivated viruses. These results demonstrate that following carbon flow facilitates identification of discrete host-virus interactions within the complex soil environment.

Keywords: Soil viruses ; DNA ; stable isotope probing ; metagenomics

Compartimentation différente de l'antibiorésistance entre phages et bactéries dans un continuum rivière - effluents de station d'épuration.

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La transduction est probablement l'un des grands mécanismes de transfert horizontal permettant à l'antibiorésistance de se disséminer entre bactéries commensales, pathogènes et environnementales. Si ce mécanisme est bien décrit *in vitro*, sa fréquence reste difficile à évaluer dans l'environnement et les paramètres et acteurs qui l'y régissent sont méconnus. Dans ce travail, nous avons étudié la compartimentation de gènes d'antibiorésistances dans les bactéries et les phages de communautés microbiennes présentes dans l'eau d'une rivière, la Meurthe, et dans les effluents d'une station d'épuration s'y déversant au cours du temps. Les ADN des bactéries et phages ont été extraits séparément en vérifiant, pour ces derniers, qu'aucun ADN libre non-encapsidé n'ait contaminé les extraits. Les ADNr 16s et un panel de 10 gènes d'antibiorésistance et 9 éléments génétiques mobiles choisis pour leur pertinence environnementale et/ou clinique ont ensuite été quantifiés par PCR quantitative ou digitale. La quantification d'ADNr 16s dans les ADN issus des fractions phagiques a permis de confirmer la présence de phages transducteurs contenant de l'ADN chromosomique dans les différentes communautés microbiennes. Si, dans les eaux de rivières, les phages transducteurs sont peu abondants et ne véhiculent qu'aux maximum 0,08% des ADNr 16s présents dans l'ensemble d'une communauté microbienne, cette valeur peut atteindre 16,3% dans les effluents de station d'épuration, démontrant ainsi la très forte proportion de phages transducteurs dans ce milieu. Cette tendance a également été observée avec les différents marqueurs suivis où, par exemple, les intégrons de classe 1 n'étaient jamais véhiculés à plus de 0,01% par les phages de communautés de rivières mais jusqu'à 2% par les phages de communautés d'effluents. Dans des cas extrêmes, des effluents ont même vu plus de la moitié des gènes *sul1* et plus du quart des gènes *vanA* être contenus dans la fraction phagique ! Globalement, la tendance présentée par les gènes d'antibiorésistance et les éléments mobiles à être encapsidé s'est avérée variable d'un marqueur à l'autre et d'un milieu à l'autre. Si les effluents étaient bien plus chargés en antibiorésistance que les eaux de rivière, aucune augmentation significative de l'antibiorésistance n'a pu être détectée dans les eaux en aval du point de rejet de la station d'épuration. La question se pose cependant de l'impact des bactéries et surtout des nombreux phages transducteurs des effluents en ce qui concerne leurs rôles respectifs dans la dissémination de l'antibiorésistance dans l'environnement.

Keywords: Transduction; Antibiorésistance; Eléments génétiques mobiles; Rivière; Station d'épuration

The role of capsules and depolymerases in Klebsiella-phage interactions

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Due to the prevalence of encapsulated bacteria, the capsule is the first barrier encountered by many phages. This layer, usually made of polysaccharides, can block the access of phages to their cell wall receptors. To overcome this, some phages have specific depolymerase enzymes (Dpos) in their receptor-binding proteins. Here, we have quantified the predictability of phage-bacteria interactions based on the capsular locus type of the host (CLT) and Dpos. For this, we have used *Klebsiella pneumoniae* (Kpn) as a model, because of its high capsular diversity and its importance in global health. We have analysed the complete interaction network of a Kpn-phage collection, comprising 138 Kpn isolates and 46 diverse phages. Spot tests revealed that the majority of phages showed capsular specificity, with their pattern of infection being accurately predicted (92%) by the CLT of the host. Consequently, phage-encoded Dpos, which have often undergone horizontal gene transfer across large taxonomic scales, were key determinants of host tropism. The identity tolerance of phage Dpos was high, as shown by the predicted capsular tropism of RefSeq prophages for 13 important CLTs. Even though capsules and Dpos predicted the first steps of virion recognition and adsorption, their accuracy dropped for productive infections (53%). Additionally, we have found four phages with a broader host range that showed both capsule-dependent and independent tropism. This was also evidenced by their ability to infect acapsular bacteria depending on the genetic background of the host. These findings expand our knowledge of the complex interactions between bacteria and their viruses and point out the feasibility of predicting the first steps of phage infection using the genome sequences of phages and hosts.

Keywords: host tropism; capsule; depolymerases; Klebsiella

Competition between phage-resistance mechanisms determines the outcome of bacterial co-existence

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Many bacterial species carry multiple prophages, and despite the potential cost, which includes cell death upon phage induction, prophages can also provide the host multiple fitness advantages, such as infection of direct competitors. However, the long-term efficiency of displacing conspecifics through prophage induction has received little attention. We experimentally coevolved a polylysogenic *Klebsiella pneumoniae*strain (ST14) with a phage-sensitive strain, BJ1, in several environments resulting in different phage infection regimes. We then followed the adaptation process and the emergence of resistance. After 30 days, population yield remained stable, and although BJ1 was present in all conditions, its frequency was higher when phage pressure was stronger. Resistance to phages emerged fast through mutations interrupting the capsule biosynthesis. In contrast to our expectation, lysogenic conversion was rare and costly because new BJ1 lysogens exhibited exacerbated death rates and were easily outcompeted. Unexpectedly, the adaptation process changed at longer time scales, where BJ1 populations adapted by fine-tuning the production of capsule, reducing the ability of phage to absorb, while remaining capsulated. These resistant clones are pan-resistant to a large panel of phages. Most intriguingly, some clones exhibited transient non-genetic resistance to phages. Our experimental and modelling results highlight the diversity, dynamics and competition between phage-resistance mechanisms during coevolution and how these are driven by phage density.

Keywords: coevolution; polylysogeny; phage resistance

Demographic coexistence or coevolution ? Epidemiological dynamics of E.Coli during infection by bacteriophages T7

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Measure, describe and predict the co-evolution of bacteria and their phages is essential for the development of bacteriophages as clinical antibacterial treatments. When studying epidemiological dynamics of bacteria/phage systems, one of the main issues is to find a precise and high throughput, yet not too laborious measurement, as a proxy of the bacterial population size over time while phage infection occurs. In our study, the production of living cells and biomass of *Escherichia coli* in the presence of bacteriophage T7 were measured in parallel on a real-time basis, using several fluoro-luminometric measurement. We evaluate the ability of the main epidemiological models of type Sensitive/Infected/Phage (SIP) found in the litterature to jointly fit these distinct measured bacterial kinetics and their mutual consistency as a test of model validity. These precise kinetics allowed us to show coexistence over long term experiments (over 100 hours) between the bacteria and the bacteriophages despite no detectable resistant bacteria. These unexpected results call for more mechanistically precise epidemiological model than the classical SIP from the litterature. Such parameterization of the epidemiological dynamic opens the perspective of predicting with good confidence the kinetics of co-evolution in bacteria/phage systems.

Keywords: host tropism; capsule; depolymerases; Klebsiella

Phage-assisted directed evolution of proteins and RNAs

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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. Transducing phage particles are able to support higher mutagenesis rates than any viral system, allowing for a faster evolution, where the host cell is re-engineered according to the desired selection. We will show how phages can be used to accelerate the directed evolution of proteins. We have engineered the genomes and hosts of phages M13, T7 and P2 to evolve proteins and RNA. For this, we have developed phage infection cycles implementing positive and negative selections. The implementation of positive and negative selections allowed the engineering of stronger activity and specificity respectively. We demonstrate the usefulness of our system by engineering the smallest transcription factor activator/repressor, a set of orthogonal transcription factors activator/repressor and a riboswitch in *E. coli*. Our methodology for accelerated directed evolution can be used to evolve any protein or RNA where its activity could be coupled to gene expression.

Keywords: synthetic biology; directed evolution

[Keynote]: Control of spore-forming bacteria by phage regulatory switches

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Phages are killers and genetic parasites, but they can also act as, so called regulatory switches (RS). RS phages integrate into functional genes inactivating them by a split, but under certain developmental stage they excise from the host chromosome allowing reactivation of the attachment genes.
The best studied example of RS phage is SPβ, a representative of Spbetavirus genus, targeting *Bacillus subtilis*. This phage integrates into *spsM* locus, involved in biofilm formation and spore coat modification. We showed that evolution under sporulation selection regime promotes recombination of SPβ with a low-copy number phi3Ts, 'hitchhiking' in certain *B. subtilis* lab isolates. This recombination results in spontaneous induction of lytic cycle, allowing the chimera phages to prey on *B. subtilis* ancestral strain. Comparing genomes of natural *B. subtilis* isolates, we discovered that Spbetaviruses are prevalent within this species, they occupy adjacent integration sites and characterize with genomic mosaicism.
Further experiments with isogenic bacterial host lysogenized with different Spbetaviruses, demonstrate strong impact of these phages on physiology and ecology of their host species, modifying features such as growth rate, cell shape, sporulation dynamics or bacteriocin production. Our results thereby show how reshuffling of genetic modules within single temperate phage genus impacts ecology and evolution of its bacterial host.

Prophage taming by the adherent-invasive Escherichia coli LF82 upon macrophage infection

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Adherent-invasive *Escherichia coli* (AIEC) strains are frequently recovered from stools of patients with dysbiotic microbiota. They have remarkable properties of adherence to the intestinal epithelium, and survive better than other *E. coli* in macrophages. The best studied of these AIEC is probably strain LF82, which was isolated from a Crohn's disease patient. This strain contains five complete prophages, which have not been studied until now. We undertook their analysis, both *in vitro* and inside macrophages, and show that all of them form virions. The Gally prophage is by far the most active, generating spontaneously over 108 viral particles per mL of culture supernatants *in vitro*, more than 100-fold higher than the other phages. Gally is over-induced after a genotoxic stress generated by ciprofloxacin and trimethoprim. However, upon macrophage infection, Gally virion production is decreased by more than 20-fold, and the transcription profile of the prophage indicates that part of the structural module is specifically repressed while the replication module is overexpressed compared to unstressed culture conditions. We conclude that strain LF82 has evolved an efficient way to “tame” its most active prophage upon macrophage infection, which may participate to its good survival in macrophages. The results are discussed in light of the active lysogeny process.

Keywords: prophage; macrophage; lateral transduction; polylysogeny; active lysogeny

YbcM, a transcriptional regulator from prophage origin involved in Escherichia coli physiology

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When integrated into the bacterial genome, temperate phages are called prophages. When expressed, genes carried by these prophages can confer advantages to their host such as increased virulence, fitness or stress resistance. However, only few studies have been undertaken to elucidate the molecular mechanisms leading to these changes in bacterial physiology.

In this work, we investigate the role of YbcM, a transcriptional regulator encoded by the DLP12 prophage in *E. coli*. By combining RNA-Seq and ChIp-Seq approaches, we identified 48 genes whose expression varies more than 4-fold when YbcM is overproduced. Our data show that YbcM directly represses 17 of them, which is odd for a regulator from the AraC/XylS family. A large number of these genes are involved in motility, adherence and biofilm formation. As a consequence, YbcM overproduction inhibits motility and promotes a particular type of biofilm called macrocolony biofilm. Interestingly, YbcM overproduction also decreases the sensitivity of different *E. coli* strains to several phages, suggesting that this surface-dependent biofilm can have a negative impact on the interaction between phages and their host.

We have also demonstrated that YbcM directly represses the expression of *crp* that encodes the central regulator of the catabolic repression in *E. coli*. This regulatory pathway is still being characterized and may have important consequences on the host physiology, particularly under glucose starvation since CRP favors the use of carbon sources other than glucose.

Overall, our results show that YbcM is integrated into the bacterial regulatory network and changes in depth the bacterial physiology. An important consequence of these changes is the immunity conferred by YbcM against potential future phage infections. This work will provide a better understanding of how prophages can genetically interact with their bacterial hosts and deeply affect their physiology.

Keywords: prophage; phage resistance; biofilm; motility; metabolism

Choreography of phages PAK-P3 and PhiKZ genomes during Pseudomonas aeruginosa infection

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If bacteria appears to be the most diverse cellular domain of life on earth, they are outnumbered by the viruses that can infect them. With an estimation of 10^31 particles, bacteriophages (phages) are the most abundant genomic entities across all habitats and a major reservoir of genetic diversity. Up to date, the vast majority of sequenced phage genomes are dsDNA and smaller than 100kb. In recent years, several publications have expanded our understanding of phages biodiversity and demonstrate the existence of phages with large genomes, rising numerous questions concerning how these genomes folds within their capsid but also during their infection cycle. Recently, a study proves the existence of a compartment that separated viral DNA from the cytoplasm in *Pseudomonas chlororaphis* phage 201phi2 and demonstrates that large phages have developed innovative mechanisms to succeed in their infection cycle. Large phages typically contain more genes implicated in genome replication, nucleotide metabolism or coding for DNA binding proteins and could, therefore, have developed new strategies concerning their 3D genome organization and the hijacking their host.

To tackle this question, we have used chromosome conformation capture (HiC) to characterize the phage-host genomes interactions during the infection of *Pseudomonas aeruginosa* by two different phages, PAK-P3 and phiKZ (PAK-P3 is a 88kb virulent phage, and phiKZ is a 280kb giant bacteriophage). We performed a kinetic of both infection cycles and followed, concomitantly, the variation of genomes architecture through time. Our data show a correlation between variations in phages genomes folding and its transcriptional program. In parallel, we observed a global disorganization in the host genome, with a decreasing of the signal of the observed borders in the genome. Our results demonstrate that phages are highly dynamics genomic entities when they are active and pave the way to in-depth analysis of their infection cycle.

Keywords: HiC; nucleus; like; jumbo phage; bacterial infection

Analysis of the microbiome and virome of a gnotobiotic mouse model by Chromosome Conformation Capture

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The gut microbiota houses a complex and diverse microbial community that is crucial for human health. Indeed, the alteration of the composition of bacteria has been associated to various chronic diseases such as inflammatory bowel disease, asthma or obesity. More recently, variations of intestinal viruses, predominated by bacteriophages (phages), have also been associated with dysbiosis, calling for combined studies of both bacterial and viral populations.

Chromosome Conformation Capture (3C) applied to microbial communities is an innovative method to obtain information on both bacterial and viral populations as well as their interactions through DNA collisions. Here, we applied this method and developed the tools to analyze both *in vitro* and longitudinal *in vivo* samples from a group of 12 bacteria stably colonizing the gnotobiotic OMM12 mice model. In addition, we performed deep sequencing *in vitro* and *in vivo* of the total viral fraction to identify the viruses naturally present in this community.

The analysis of data from the 3C method led us to improve the assembly of the 12 bacterial genomes and revealed the precise 3D structures of their chromosomes, providing novel information on the diversity of architecture of non-model bacterial chromosomes and the metabolic activities of these bacteria in the gut environment. In particular, we detected the 3D signature of prophage induction amongst which several formed free particles as confirmed by virome sequencing. This result demonstrates that 3C data can discriminate functional prophages from cryptic ones. The comparison between *in vitro* and *in vivo* data also led to the observation that the gut environment impacted both prophage induction as well as the 3D structure of the bacterial genome. Finally, the temporal stability of bacteria and phage populations was assessed over time as well as the reproducibility of the method.

Altogether, these data demonstrate that the combination of virome and *in situ* 3C data can reveal the dynamic interactions between phages and bacteria. These results provide a solid base for implementation to further study microbial communities in the gut environment using this gnotobiotic model.

Keywords: Gut microbiota; Chromosome Conformation Capture; Virome; Mouse model; Prophage induction

Membrane-less compartimentalization in time and space of bacteriophage SPP1 replication and assembly in the Gram-positive bacterium Bacillus subtilis

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Viral infection affects host cell homeostasis and draws extensive cellular biosynthetic resources. Cell machineries are also hijacked for optimal viruses multiplication. Here, we investigated the impact of these processes in the spatial organization of the bacterial cell. We show that infection by bacteriophage SPP1 leads to the formation of two types of membrane-less compartments in the cytoplasm of the Gram-positive bacterium *Bacillus subtilis*.

Phage DNA localizes in a single DNA compartment in mono-infected cells. More than 300 copies of the SPP1 viral genome are synthesized in the first 25 minutes of infection, doubling the cell DNA content. This process requires fast recruitment of the bacterial replisome proteins orchestrated by the SPP1 helicase gp40 that binds to the DnaG primase and to DnaX, a subunit of DNA polymerase III. Hybrid phage-bacterial replisomes accumulate in discrete genome replication factories within the phage DNA compartment. Collectively, our data reveal that the host replisome machinery is massively redirected and dedicated for optimal SPP1 DNA replication.

Later in infection, procapsids localize at the periphery of the DNA compartment for genome packaging whereas DNA-filled capsids fully segregate to spatially distinct warehouse compartments where viral particles accumulate. Warehouses are found mostly side by side from the viral DNA replication *foci*.

The dynamics of the SPP1 replication factory and virions warehouses were visualized during the complete SPP1 infection cycle using microfluidics. The spatial and temporal distribution in the bacterial cytoplasm highlights a sequential program of molecular interactions. It leads to an extensive re-organization of the crowded cytoplasm to achieve assembly of about 150 infective particles within 25 minutes of infection. Structuration of viral factories to confine phage DNA enzymatic reactions appear as a very efficient strategy for SPP1 to exploit the bacterial resources for its own profit.

Keywords: Viral factories; virions warehouses; membrane-less compartments; DNA replication; particles assembly; video; microscopy

Structural basis of cell wall perforation by bacteriophage T5

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The vast majority of bacteriophages (phages) - bacterial viruses - present a tail that allows host recognition, cell wall perforation and safe channelling of the viral DNA from the capsid to the cytoplasm of the infected bacterium. The majority of tailed phages bears a long flexible tail (*Siphoviridae*) at the distal end of which a tip complex, often called baseplate, harbours one or more Receptor Binding Protein·s (RBPs). Interaction between the RBPs and the host surface triggers cell wall perforation and DNA ejection, but little is known on these mechanisms for *Siphoviridae*. Here, we present the structure of siphophage T5 tip at high resolution, determined by electron cryo-microscopy, allowing to trace most of its constituting proteins, including 35 C-terminal residues of the Tape Measure Protein. We also present the structure of T5 tip after interaction with its *E. coli* receptor FhuA reconstituted into nanodisc. It brings out the dramatic conformational changes underwent by T5 tip upon infection, *i.e.* bending of the central fibre on the side, opening of the tail tube and its anchoring to the membrane, and formation of a transmembrane channel. These new structures shed light on the mechanisms of host recognition and activation of the viral entry for *Siphoviridae*.

Keywords: Phage T5; Structure; Perforation de la paroi bactérienne; microscopie électronique

Raising the bar in undergraduate teaching: discovery and characterization of phages infecting Corynebacterium glutamicum by Bachelor students at Paris-Saclay University

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Inspired by the SEA-PHAGES program established for 14 years in many American universities (1), two undergraduate teaching units (40 h each) were created at the University Paris-Saclay in 2021-2022 to isolate and characterize phages infecting *Corynebacterium glutamicum*. *C. glutamicum* belongs to the Gram positive but didermic Actinobacteria for which few phages have been characterized compared to Mycobacteria. In the first teaching unit called "Phage Discovery", thirteen 2nd-year Bachelor students* collected environmental soil and water samples. Using a strain of *C. glutamicum* devoid of prophages for enrichment and purification steps, the students isolated two distinct bacteriophages, observed their morphology by electron microscopy and analyzed their DNA by restriction digests. In preparation for the second teaching unit "Phage DNA explorer", viral DNA was sequenced. Then, 23 3rd-year Bachelor students* analyzed the assembled viral genomes using GLIMMER, BLAST, HHblits and InterPro.

The first isolated phage, named CyranoPS, exhibited a 90-nm long flexible tail twice as long as its capsid diameter. Analysis of its 15-kb genome revealed 22 protein coding sequences (CDS) of which 64 % were assigned a function (virion assembly, host cell lysis, transcriptional regulation, lysogeny). Consistent with the production of turbid lysis plaques, its genome encoded a predicted integrase, suggesting a lysogenic lifecycle.

The second phage JeanGrey presented a large capsid diameter (96 nm), a 91-nm contractile tail and a large genome (106 kb). Functions were predicted for 37% of the 155 CDS. Besides virion assembly and host cell lysis, the function of 18 % of CDS was ascribed to DNA metabolism, including a DNA polymerase and a deazaguanine DNA modification system. Production of small clear lysis plaques and the absence of integrase indicated that JeanGrey is a virulent phage.

Taken together, our results show the successful creation of an undergraduate classroom research experience at a French university. Further implementation in France and Europe could be developed in a network of higher education institutions in partnership with research labs interested in phage ecology, genomics and evolution.

(1) Jordan et al. 2014. mBio 5(1):e01051-13. doi:10.1128/mBio.01051-13.

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Keywords: Undergraduate classroom research experience; Corynebacterium; Genome annotation

[Keynote]: Beyond boom and bust: re-evaluating the history of West-European phage therapy

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The history of bacteriophage therapy is traditionally told as a story of booms and busts: following an interwar high, the antimicrobial era triggered a precipitous decline of therapeutic demand in Western countries – despite ongoing popularity East of the Iron Curtain. But how accurate is this account? And what can studying the ongoing circulation of bacteriophages tell us about challenges facing current therapy efforts? In this presentation, I draw on previously unstudied records at Institut Pasteur to map the persistence of large-scale therapy networks in post-war France. Findings highlight the importance of distinguishing between therapeutic markets in North America and Western Europe, reveal the long-term challenges facing phage therapy, and demonstrate the usefulness of engaging with neglected phage-archives in the genomic era.

PHAGEinLYON / PHAG-ONE : Optimization of pharmaceutical production of anti-S. aureus therapeutic phages using in silico and experimental approaches

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Introduction. Treatment of multi-drug resistant bacteria is a major health challenge for the next decade. Phage therapy is a promising therapeutic alternative to antibiotics and the "Hospices Civils de Lyon" (HCL) have acquired a significant clinical and microbiological experience in this field. Through PHAGE*in*LYON program, and PHAG-ONE project, HCL are implementing the first public platform for production of therapeutic phages. After one year of development, a bank of 17 phages anti-*S. aureus* was built and 3 of these phages were selected for their high therapeutic potential. The development of pharmaceutical production processes of these 3 therapeutic phages must address several major challenges: i) the risks associated to the use of a pathogenic bacterial strain for phage production inducing potential contamination of therapeutic products by virulence/resistance factors or lysogenic phages, ii) the need for high production yield suitable for clinical use. Here, we report the development of these processes in compliance with regulatory agency requirements.

Methods.

i) We performed an *in silico* analysis of bacterial genomes of the large collection (n>2000 genomes) of the Centre National de Référence des Staphylocoques using a proprietary algorithm/software to select the strains best suited for phage production by excluding all bacteria harboring major virulence /resistance factors and temperate phages.

ii) Experimental parameters (bacterial growth media, inoculum ratio phage/bacteria, kinetic of phage production) were optimized in a pharmaceutical scale up.

Results.

i) With the *in silico* approach, we selected 6 *Staphylococcus aureus* candidate strains, over more than 2000 clinical strains. Only 3 of them allowed to reach an amplification yield acceptable for pharmaceutical production.

ii) Production optimization experiments allowed us to identify amplification conditions in a medium usually used to industrially produce recombinant proteins, to obtain phage titers ≥ 1.1011 PFU/mL in only 4 hours for the 3 selected therapeutic phages.

Conclusion. We report here the first steps of the pharmaceutical process for therapeutic phage production in compliance with regulatory requirements. The next ongoing step is the development of purification processes and specific quality controls to ensure the safety of therapeutic phages.

Keywords: Phage production; optimization; yield; safety; Staphylococcus

Directed in vitro evolution of virulent bacteriophages against Enterococcus faecium following the Appelmans Protocol

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Enterococcus faecium is a ubiquitous Gram-positive bacterium that commensally colonizes the human intestinal tract. It is also an opportunistic pathogen that has become a major cause of nosocomial infections worldwide. This is related to the emergence of clinical isolates belonging to the clonal complex 17 (CC17), which are multiply resistant to antibiotics and particularly vancomycin (the so-called vancomycin-resistant enterococci (VRE)). Phage therapy is a way to combat multidrug-resistant bacteria, including VRE. For that, the productive host spectrum needs to be broad enough. However, we and others found that this was not the case for phages infecting *E. faecium*. We therefore attempted to generate « generalist phages » active on a wide panel of CC17 clinical isolates.

To this end, we used a protocol recently developed by Burrowes and his colleagues, inspired by Georgian researchers and designated the Appelmans protocol. It consists in the iterative growth of a serially-diluted phage cocktail on a set of naive related strains, most of which were initially refractory. Phages from the evolved cocktail were then isolated and their individual host spectrum evaluated to find « generalist candidates ». The experiment was conducted with a phage cocktail composed of four well-characterized CC17 bacteriophages from our collection: one myophage Porthos belonging to *Shiekvirus* genus and *Herelleviridae* family, one siphophage Planchet from the *Denvervirus* genus and two related siphophages dArtagnan and Aramis representing a new genus. Fifteen successive passages of the cocktail were performed using eight VRE strains, of which five were initially refractory to at least one of the phage from the cocktail. Eighteen phages were isolated and purified, and their host ranges were analyzed on a total of 14 CC17 isolates. Genomes of four evolved phages, with a more extended host spectrum, were sequenced, assembled and analyzed. Three phages corresponded to Porthos mutants that were active against 10 of the 14 strains, representing a spectrum twice as large as the WT Porthos. Three point mutations in the tail module of the evolved Porthos could at least partly explain their extended host ranges. The fourth mutant was an Aramis recombinant, in which a DNA region of the tail module had been acquired from dArtagnan. This mutant was active against five of the 14 strains, which represented two additional targets compared to the WT Aramis. These results are encouraging and illustrate that the Appelmans approach is an elegant way to enlarge host spectrum of virulent phages.

Keywords: Enterococcus faecium; virulent bacteriophage in vitro directed evolution; extended host spectrum

Evolutionary training: how bacteriophages adapt to multiple strains of Salmonella enterica

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The efficacy of phage therapy depends not only on the ability of bacteriophages to infect pathogenic bacteria but also to limit the emergence of new resistant bacterial genotypes nor to select rare resident resistant genotypes. We performed *in vitro* evolutionary training of one isolated and purified Tequintavirus bacteriophages (four independent lineages) by performing 6 to 7 consecutive passages against 8 not co-evolving bacterial genotypes *of Salmonella enterica* serotype Tenessee. While the ancestral bacteriophage was able to infect 3 out of 8 bacterial genotypes, evolved populations expanded their host range (8/8 infected bacterial genotypes). Moreover, bacterial growth inhibition of adapted bacteriophage populations was maintained without appearance of resistant bacteria for more than 20 hours despite a 3-4 log dilution of the bacteriophages. After sequencing DNA from both ancestral and evolved populations, we observed parallel evolution toward modification of several genes such as the long tail fiber protein gene (potentially involved in host range expansion) and exo- and endo-nuclease as well as hydrolase (potentially involved in increase of virulence). For the sake of successful phage therapy, our results demonstrate the importance of *in vitro* evolutionary training taking into account the diversity of bacteria isolated *in situ* prior to the use of therapeutical bacteriophages.

Keywords: Adaptation; biocontrol; Bacteriophage evolution; coevolution; experimental evolution; host range; Phage Therapy; Salmonella enterica; Sequencing

Evaluation of the prophylactic and therapeutic effect of a phage cocktail to control Salmonella Enteritidis in poultry

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Salmonella is one of the main causes of foodborne diseases related to poultry product consumption and is a public health concern. The use of lytic bacteriophages could be a novel, safe and effective approach, to reduce the prevalence of *Salmonella* in poultry and subsequently reduce the incidence of foodborne salmonellosis in humans.

This study evaluates the prophylactic and therapeutic effect of phages, administered via drinking water, on *Salmonella* levels in chickens. A cocktail of 6 lytic phages with demonstrated *in vitro* efficacy against various *Salmonella* serotypes was used.

First, the phages were demonstrated to persist in chickens' gut for at least 3 days without *Salmonella* challenge. The prophylactic potential of the cocktail was then evaluated *in vivo.* Fifty chicks were challenged by oral gavage with *Salmonella* Enteritidis LA5 at 5x104 CFU/chick at 7 days of age. Phages were administrated before the challenge via drinking water during the first 6 days of the chicks' life and 2 days prior to the end of the trial to check emerging resistance. The ability of resistant clones to colonize chicks was also studied. The therapeutic potential of the cocktail was also evaluated by giving phages at the end of the trial to decrease carcass contamination at slaughter. During the different trials, *Salmonella* enumeration and phage identification and counting were investigated.

Results showed that up to 4 days post infection, phages had a preventive effect and they significantly ($P<0.05$) reduced *Salmonella* colonization in ceca and feces by 2 to 4 logs. *Salmonella* levels increased 7 days post infection, after phage treatment was stopped. During this period, only 2 out of 6 phages were detected in the different gut segments. *Salmonella* resistance was observed only against these persistent phages and resistant clones recovered shown a reduced colonization capacity in chickens' gut. No resistance was observed against the other phages. Indeed, resumption of phage administration 2 days before the end of the trial reduced the *Salmonella* loads again by 1 log in the ceca. In parallel, the therapeutic use of phages reduced *Salmonella* cecal level by 4 logs compared to the situation before phage administration.

This treatment showed encouraging results regarding the effect of phages on *Salmonella* levels in chickens during critical steps of poultry production. Future work will be to study the mechanisms of resistance acquired by *Salmonella in vivo* and also the impact of this treatment on the chicken's microbiota.

Keywords: Salmonella; Poultry; phage therapy

Adaptation of the Group A Streptococcus bacteriophages to the human host

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Streptococcus pyogenes, or Group A Streptococcus (GAS), is responsible for mild (pharyngitis) to life-threatening (necrotizing fasciitis) infections. A resurgence in severe invasive infections and scarlet fever has been observed since the 1980s. Population genomic studies have highlighted the key role of bacteriophages, which carry virulence genes, and horizontal gene transfer in the emergence and expansion of epidemic lineages. Despite this, little is known about the biology of GAS phages. In addition, after a century of vaccine development, we still rely on antibiotics to treat GAS infection. Success of new strategies, like phagotherapy, also requires the understanding of the tripartite phage-pathogen-host interactions. To decipher phage-GAS interactions, we used RNAseq to explore the changes in transcriptome during infection by the virulent phage A25. We found a reprogramming of up to 33% of the transcriptome in a susceptible M25 strain. The most downregulated genes belong to the fatty acid synthesis (FASII) pathway. In presence of human serum, GAS is known to both downregulate this pathway and use fatty acids (FAs) bound to albumin (HSA). Moreover, GAS is able to shield from the human host immune system through the binding of serum proteins. At physiological concentration, we showed that the serum also protects GAS from phage A25 infection. In contrast, addition of HSA+FAs fasten the collapse of the bacterial population compared to HAS alone. We next quantified the FAs content of GAS and found a lack of C18:2 FAs, which can be provided by HSA+FAs and decrease by 21% during phage infection. Moreover, adding linoleic acid (C18:2) to HSA-FA restored the faster collapse of the bacterial population. The phage A25 originated from a temperate phage by loss of integrase and repressor genes. Therefore, the ancestor may have evolved to use F.As to compensate for the negative effect of serum on its infectivity. To determine if this applies to other prophages, we used the functional prophage φM1 integrated in a M1 strain. Interestingly, we found that the serum rather increases the lytic growth of φM1 during infection of a highly virulent M1T1 clone, ultimately leading to an increase in the proportion of potentially even more virulent lysogens. Altogether, these results highlight the need to study phage-pathogen interactions in a more physiological context. While the virulent phage A25 seems not a good candidate to treat GAS infection, turning virulent the phage φM1 could be a promising strategy.

Keywords: Streptococcus pyogenes; Temperate & lytic phages; Transcriptomic; Human serum; Fatty acids

Investigations of the role of neutrophils and macrophages in immunophage synergy during experimental pulmonary phage therapy

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In an era of unprecedented emergence of antibiotic resistant bacteria, alternative therapeutics are being urgently sought. There is a renewed interest in bacteriophages - viruses that replicate within and lyse bacteria cells - therapy to combat bacterial infections. However, the mechanisms underlying phage-medicated bacterial clearance in an animal host remain unclear. In previous work, we showed using a murine model and infection by *Pseudomonas aeruginosa* that synergy between bacteriophages and the immune system was necessary to clear bacterial infection. Specifically, severe neutropenia by injection of an anti-GR1 antibody leads to treatment failure, pointing to a primary role for neutrophilic polynuclei (NPCs)(Roach et al., 2017). In this work we aimed to determine the threshold of neutrophils required for an efficient synergy with bacteriophages and to investigate the role of alveolar macrophages during the treatment. Neutrophil depletion resulted in a rapid progression of infection relative to the non-depleted control. Furthermore, the level of neutropenia correlated with the severity of infection (R=0.8): the lower the level of neutrophils was at infection the higher the bacterial load was at the end of the infection. Bacteriophage treatment of neutropenic mice lowered the bacterial load but did not clear the infection within the time frame of our experiments. There was no correlation between the level of neutrophils at infection in the depleted mice and the efficacy of phage treatment. Infection of the macrophage depleted mice appeared to be more severe than the control mice (as derived from recording of bacterial luminescence in the lungs during infection), however, we did not find statistical evidence that macrophage depletion had an effect on the CFU levels of *Pseudomonas aeruginosa* at sacrifice. Phage treatment of the macrophage depleted mice was successful at clearing infection (no CFUs detected at sacrifice). Surprisingly, phage treatment was more effective in macrophage depleted mice than in the control. This indicates that the macrophages somehow interfere with phage mediated bacterial clearance by a mechanism that remains unclear for now.

Roach, D.R., Leung, C.Y., Henry, M., Morello, E., Singh, D., Di Santo, J.P., Weitz, J.S., Debarbieux, L., 2017. Synergy between the Host Immune System and Bacteriophage Is Essential for Successful Phage Therapy against an Acute Respiratory Pathogen. Cell Host Microbe 22, 38-47.e4. https://doi.org/10.1016/j.chom.2017.06.018

Keywords: Phage therapy; neutrophil depletion; macrophage depletion

Face à l'antibiorésistance, une écologie politique des microbes

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Durant les cinq dernières années, j'ai observé, en tant qu'anthropologue des sciences et de la santé, le développement de la phagothérapie en France après pourtant plus de cent années d'existence. Des pratiques de laboratoire aux récits des personnes malades, en passant par les dédales de la réglementation sur les médicaments et les produits de santé, les essais cliniques et la place des virus bactériophages dans les différents écosystèmes, j'ai essayé de comprendre, avec les biologistes, les médecins, les associations de malades et les agences de réglementation, comment et sous quelles conditions les phages pouvaient devenir des alliés dans la lutte contre la résistance bactérienne aux antibiotiques en santé humaine. Le résultat de ce travail est un ouvrage dans lequel j'esquisse les contours d'une médecine qui existe déjà discrètement, attentive à la dimension écologique des infections, dans laquelle les phages auraient toute leur place, ainsi que les conditions de possibilité d'une telle pratique de l'infectiologie. Je montre notamment comment il est impossible de penser le développement des phages en santé humaine sans prendre en considération la façon dont les antibiotiques ont profondément transformé non seulement la médecine et la pharmacie dans la seconde moitié du 20ème siècle, mais plus globalement l'ensemble des modes de vie et des sociétés sur Terre, de façon directe ou indirecte. Les antibiotiques, molécules chimiques présentées comme miraculeuses, ont été et sont toujours produits puis consommés en masse, en santé humaine mais bien plus encore dans l'agro-industrie. Ils sont devenus, par leurs multiples et souvent malheureux usages, un véritable pharmakon : tout autant un remède qu'un poison. Il s'agirait de ne pas reproduire les mêmes erreurs avec les phages. La phagothérapie, dès lors, ne peut constituer une réponse pérenne, bien que partielle, aux problèmes posés par la résistance aux antibiotiques, que si elle s'invente sur des fondements radicalement différents de ceux de l'antibiothérapie. Elle pourrait alors permettre de soigner des infections causées par des bactéries résistantes tout en préservant l'efficacité des antibiotiques disponibles et à venir ainsi que les bactéries non pathogènes dont les études sur le microbiote ne cessent de nous démontrer l'importance pour la santé humaine. Des initiatives sont déjà à l'œuvre, que ce réseau m'a permis d'explorer.

Keywords: anthropologie; antibiorésistance; économie; production des savoirs; interdisciplinarité

Poster Presentations

[Poster 1]: Do phages of Pseudomonas syringae communities reflect the ecology and diversity of bacteria in apricot trees?

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The distribution and diversity of phages in the biosphere places them in key roles within their ecosystems. Phages influence their bacterial host populations in many ways, from their population size to driving their genetic diversity and pathogenic potential. Unlike the aquatic or intestinal environments, the dynamics governing the interactions between phages and bacteria in agriculture are poorly investigated. *Pseudomonas syringae* is a species complex harboring a great genetic diversity of strains that are widely distributed in many environments. In the context of an apricot orchard, a multitude of genetically distinct strains of *P. syringae* can be present depending on the substrate and the season, with some strains inducing the devastating apricot bacterial blight disease. This study aims to identify the phage populations of *P. syringae* strains associated with the different apricot trees substrates (soil, branches, buds, leaves) and to test their relationship with the bacterial diversity and the disease incidence . In a first step of the study, we collected more than 200 *P. syringae* phages from soil samples in apricot orchards in the south of France, suggesting a prevalence of phages in this substrate. We are currently identifying them by sequencing their genomes and we plan to analyze some phenotypic traits such as host range. Using this phage genomic reference data base, we will then perform metagenomic analysis of phages and bacteria in the different ecological niches. This approach will allow the identification of the prevalence and diversity of phages of *P. syringae* and how it interacts with its bacterial host population in different microhabitats.

Keywords: Bacteriophage ecology; Pseudomonas syringae; Agriculture

[Poster 2]: Divergence in bacterial ecology is reflected by difference in population genetic structure, phage-predator load and host range

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Phages depend on their bacterial host to replicate, but how habitat, density and diversity of the host population drive phage ecology is not well understood. Here, we address this question by comparing two populations of marine bacteria and their phages collected during a time series sampling in an oyster farm. *V. crassostreae* reproduce more specifically in oysters. This population is genetically structured into clades of near clonal strains favoring multiple infections by closely related phages and leading to a modular structure of the phage-bacterial infection network. *V. chagasii*, on the other hand, first blooms in the water column from where it colonizes oysters through filter-feeding. We find a higher phage predation pressure on *V. chagasii* that does not result from a broader host range of the phages but rather from a greater burst size generating more infectious particles in the environment. Smaller modules in phage-bacterial infection network are consequence of a lower number of near clonal *V. chagasii* hosts as well as a higher diversity of the associated phages, which display a higher degree of specialization. We further highlight a role of blooms in generating large phage numbers, epigenetic and genetic variability. We speculate that these blooms play a major role in phage adaptation to host defense systems.

Keywords: Natural population of vibrio; Phage ecology; Bloom

[Poster 3]: Repurposing a DGR-containing bacteriophage to study non-amenable Bacteroides

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Diversity-generating retroelements (DGRs) are molecular systems that introduce high-frequency sequence variability into target regions of bacteriophage and bacterial DNA. 1,2 These systems often target tail-fiber genes of phages, diversifying their host tropism and increasing their possibility of infecting new hosts3. One of such bacteriophages is the Hankyphage4, a remarkably widespread *Bacteroides* prophage containing a DGR that was identified bioinformatically but whose DGR-induced effect on host tropism was not explored. Moreover, *Bacteroides* are prominent members of animal microbiomes5 whose study, especially of non-model strains, is currently hindered by a lack of genetic tools and a natural resistance to transformation and conjugation. This project aims to better understand the lytic-lysogenic cycle of the Hankyphage as well as the extent and ecological relevance of its DGR-driven host tropism among *Bacteroides*. Additionally, through directed evolution and genetic enhancement of the lytic cycle we hope to expand the Hankyphage host range and create an adaptable tool for the introduction of new genetic traits into non-genetically amenable *Bacteroides* strains in order to study their microbiome-associated functions.

1. Guo *et al.,* 2014
2. Roux *et al.,* 2021
3. Liu *et al.,* 2002
4. Benler *et al*., 2018
5. Eckeburg *et al*., 2005

Keywords: Diversity Generating Retroelements; Host range expansion; Genetic tool; Microbiome; Bacteroides; Hankyphage

[Poster 4]: Characterization of a T4-related vibriophage with broad host range

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T4-giant phages attract much attention due to their large genome size and gained interest as models for exploring the molecular mechanisms of broad host range. During a 5-month time series aimed at investigating the dynamics of phage–bacteria coevolution in natural populations of an oyster pathogen, *Vibrio crassostreae,* and its phages, we isolated, out of 243 vibriophages, one phage (6E35.1a), showing a broad host range with 21 sensitive *V. crassostreae* isolates. Phylogenetic analysis indicated that this phage (253-kbp) had the same classification as KVP40, a T4-like phage of the genus *Schizotequatrovirus*.

One-step growth curves showed that 6E35.1a has a relatively long latency time of 35 min and a reduced burst size of ~7 PFU/cell. To determine viral host range, we challenged the 21 *V. crassostreae* strains, described as sensitive by spot testing, in liquid assays and quantified viral progeny production by qPCR. Out of the 21 tested hosts, 10 strains allowed very low or no production of virus progeny. We characterized for these strains the mode of resistance by testing for attachment using adsorption assays. Phage attached to all 10 hosts, indicating that resistance was intracellular in all interactions tested. Because a 27-kDa outer membrane protein, OmpK, serves as receptor for KVP40, we next generated an *ompk*-knockout mutant in the original host of phage 6E35.1a. The *ΔompK* mutant was not sensitive to phage infection nor allowed phage adsorption. When constitutively expressed in trans from a replicative plasmid, phage adsorption to the mutant and sensitivity to phage infection were restored, demonstrating the role of OmpK as receptor. Finally, we isolated 10 spontaneous phage-resistant variants. To investigate whether the mutations affected *ompK* or non-mutational defense mechanisms, these variants were complemented *in trans* with the *ompK*-expressing plasmid and subjected to phage infection. All transconjugants recovered susceptibility to 6E35.1a, indicating that *ompK* mutations are responsible for phage resistance.

Importance: We show here that two T4-giant phages (KVP40 and 6E35.1a) target the same receptor, suggesting that generalism results from phages having evolved the ability to attach to conserved cell-surface molecules rather than from phages attaching to different cell-surface molecules in distinct hosts. We also report that resistance to this phage relies on intracellular defense mechanisms, as described for generalist cyanophages. We will next investigate the transcriptome dynamics of one or two host-phage pairs to catalog host and phage genes necessary for the interaction and assess the molecular mechanisms underpinning infection by this phage.

Keywords: broad host range vibriophage T4-like phage

[Poster 5]: Erwinia phages as biocontrol tools against fireblight disease in fruit trees

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Fireblight is one of the most important fruit diseases and has catastrophic consequences for apple and pear trees. The development of an innovative strategy for an effective fight against it is a priority challenge for fruit production in France and worldwide. This disease is caused by the bacterium *Erwinia amylovora* and a potential successful biocontrol strategy could be the use of phages, the viruses of bacteria. To achieve this goal, a collection of 16 new Erwinia phages isolated in the south of France were analysed to determine their morphology, their genome and their host range on a selection of 44 *E. amylovora* strains. This phage collection represents 5 genera, including 1 new, and 7 different species, including 4 new ones. The phages observed are tailed, with a majority of myovirus and a few podovirus morphological types. Phage lifestyle analysis determined that all phages are virulent, as reported before for Erwinia phages. Ten phages showed a large host range, targeting more than 20 *E. amylovora* strains, and 2 phages were capable of infecting 100% of the strains. A selection of Erwinia phage candidates based on genomic diversity and host range extent criteria was made for subsequent tests. Currently, the efficacy of the selected phages in controlling the bacterium *in vitro* and on apple tree seedlings is under evaluation. Overall, this project seeks to prove the potential of phages as an efficient biocontrol tool against fireblight disease

Keywords: Fireblight; Erwinia amylovora; bacteriophage biocontrol; Agriculture

[Poster 6]: Insight into key structural points of capsule-targeting phage depolymerases specific to Klebsiella pneumoniae K63

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Phage depolymerases are enzymes capable of degrading bacterial capsules, making cells accessible for antimicrobials and the immune system. Depolymerases are a highly interesting anti-virulence tool against encapsulated *K. pneumoniae* (WHO priority 1 pathogen - CRITICAL). Apart from reports regarding the biological activity of depolymerases there is not much data available on structural characteristics with particular emphasis on the catalytic centre. Those are generally large proteins (>50kDa), forming trimers characterized by a modular organization with various stability features. We intended to prepare ”mini-enzymes” (truncated versions with a catalytically active domain but lower MW and more stable). Here we present two depolymerases encoded by *Klebsiella*-specific phages KP34 (KP34p57), and KP36 (KP36p50), both active against capsular type K63. The structural prediction done using Artificial Intelligence (AlphaFold software) allowed us to identify the crucial amino acid residues of the catalytic pocket of both enzymes, a finding which we verified experimentally by site-directed mutagenesis. Also, it allowed us to design a spectrum of truncated forms, with different stabilities and catalytic properties.
We believe that our results will facilitate the identification of catalytic sites of other depolymerases in the future and allow for the selection of the appropriate truncation positions for such enzymes. These could be applied for the preparation of active mini-enzymes with preserved enzymatic functions.
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Keywords: bacteriophage; polysaccharide depolymerase; Klebsiella pneumoniae

[Poster 7]: Moleculars mechanism of phage MDAΦ entry in Neisseria meningitidis

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Neisseria meningitidis is a commensal bacterium that colonizes the human nasopharyngeal mucosa. Under ill-defined circumstances, the bacterium crosses the nasopharyngeal barrier and spreads in the bloodstream. A filamentous phage, designated MDAΦ for Meningococcal Disease Associated, has been associated with invasive meningococcal diseases in young adults (Bille et al. 2005; 2008). This phage can infect different meningococcal strains using their type IV pili (TFP) and hijacks the TFP secretin PilQ to be extruded without damaging the host (Meyer et al. 2016). Biofilm analysis revealed that phage production in place of TFP maintain bacteria-bacteria interactions favoring bacterial colonization onto a monolayer of epithelial cells. This suggest that MDAΦ increase the occurrence of diseases by increasing bacterial colonization in the mucosa at the site-of-entry (Bille et al. 2017).
Our aim is to understand the molecular mechanism by which MDA enters the host periplasm. We focused on examining the interaction between the phage and TFP. Investigations using deletion mutants of genes involved in the TFP machinery showed that phage entry requires functional and retractable TFP. We then focused on the major pilin PilE. We constructed a PilE phylogeny based on genomes available on PubMLST, which shows that the PilE sequence of prophage-containing isolates appears to be clustered. This result suggests that phage entry may dependent on the sequence of PilE, which is subject to antigenic variation. To support this hypothesis and determine the mechanism involved in PilE/MDA interaction, we experimentally tested MDA entry in strains expressing various *pilE* sequences. We identify PilE sequences allowing or not MDA entry. Finally, structure prediction and analysis of the charged amino acids of TFP and those of MDA confirm our hypothesis. Together, our data support a new model of interaction between filamentous phage and type IV pili.

Keywords: Neisseria meningitidis; filamentous bacteriophage; type IV pili; interaction

[Poster 8]: Current and future clinical phage product quality control in Belgium

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As antibiotic resistance become a world health issue, new interest in phage therapy has risen all around the world. However phage therapy is not practiced the same way everywhere: Contrary to the rest of the world, in Georgia, Poland and Russia, phage therapy was never abandoned and is still being used routinely. Access to phage therapy is not limited by state agencies, and in Russia, over-the-counter phage products are sold in pharmacies as a registered product. In most of other countries, phage therapy is only considered as a last resort treatment for "compassionate use", with oversight by the state drug agency (*e.g.* ANSM in France, or the FDA in the US). On the other hand, in Belgium, phage therapy has a special status as phages are considered "Active Pharmaceutical Ingredients" (APIs) and as such can be prepared and combined according to a monograph by an hospital pharmacist. This special status has made Belgium the most active country in the EU regarding phage therapy. This monograph states that clinical phage product (API) have to be prepared in a "GMP grade" room (Queen Astride Military Hospital) and their quality controlled by a state mandated laboratory (Sciensano). This control is divided in two parts: the phage passport (looking at specific genomic markers *e.g.* virulence, lysogeny genes) and the phage product QC (looking at biochemical properties *e.g.* pH, endotoxins). All of this process insure the quality and safety of the product and prevented the administration of contaminated products to patients.
Overall, we will present this phage product quality control process in details, acknowledge its limitations and also discussed its evolution in the future.

Keywords: Phage Therapy; Safety; Quality Control

[Poster 9]: Evolution and conservation of antiviral mechanisms between Prokaryotes and Eukaryotes

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Viruses are a constant threat to both prokaryotic and eukaryotic cells. The arms race between cells and viruses has led to innovation and diversification of anti-viral mechanisms. A consequence of this evolutionary trend is that phylogenetically distant organisms across Kingdoms encode different and specific antiviral mechanisms. For example restriction-modification and CRISPR-Cas systems are only present in prokaryotes. Recent discoveries in the field of anti-phage defense challenged this view, as it was uncovered that multiple eukaryotic antiviral systems have prokaryotic counterparts including TIR domains, cGAS, viperins and gasdermins. Here, we trace the evolutionary history of the eukaryotic anti-viral systems inherited from prokaryotes by employing bioinformatics analysis combined with experimental methods. We characterize the antiviral arsenal across eukaryotic proteomes and demonstrate that specific anti-viral systems have been transferred from prokaryotes to eukaryotes during or after the emergence of the eukaryotes. Focusing on the evolutionary history of viperins, we explore molecular transitions marking the emergence of enzymatic specificities of the eukaryotic versions of such systems. Finally, we extend our observations from viperins to other anti-viral systems allowing us to propose a scenario for the evolution of prokaryotic anti-viral systems to eukaryotic ones. Our results contribute to our understanding of the emergence of the Eukaryotic antiviral immune system.

Keywords: Evolution; Anti phage defense; Viperins; Eukaryotes; Prokaryotes

[Poster 10]: Determining the scale and rates of adaptation by methane-oxidising bacteria in response to in situ virus interactions

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We have considerable knowledge of virus-host interactions and evolution in a variety of ecosystems including marine water, wastewater and the human gut. However, in the soil environment, investigating virus-host interactions between individual populations is challenging due to the vast diversity of both prokaryotes and viruses in addition to the physicochemical complexity. The recent development of viral bioinformatic tools in conjunction with metagenomic approaches has enabled description of the diversity and complexity of virus communities in soil. However, we do not yet understand the dynamics of host-virus interaction or the rates of evolution of hosts in response to virus infection. We have recently developed an approach using 13C DNA stable isotope probing that enables analysis of the transfer of assimilated carbon from host to virus *in* situ. Using 13C-enriched methane to focus on a taxonomically and functionally restricted group of organisms, we identified native strains of methane-oxidising *Methylocystis* that grow in soil microcosms under defined incubation conditions (25oC, 10% methane headspace concentration). Subsequently, using long-range PCR and MinION metagenomic sequencing, we have developed an assay that targets the clustered regularly interspaced short palindromic repeats (CRISPR) arrays of *Methylocystis* populations to examine the impact of virus interaction and CRISPR array evolution under different conditions. Specifically, we aim to test the hypotheses that growth in soil results in virus infection and subsequent integration of novel spacers in *Methylocystis* CRISPR arrays during short-term incubations, and these rates of integration are dependent on growth rate and activity.

Keywords: Methylocystis; CRISPR array; Methane; host; virus interaction

[Poster 11]: A F4 cluster Mycobacteriophage Ritam007 with biofilm inhibitory activity and a unique lysis cassette

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Mycobacteriophages are phages that infect *Mycobacterium spp*. Due to the high diversity in their genome sequences, mycobacteriophages are divided into clusters and sub-clusters. Cluster-F is classified into five subclusters and phages and encoded lysins from this cluster have been demonstrated to have potent anti-mycobacterial properties. This study describes the characterization of an F4 sub-cluster mycobacteriophage *Ritam007,* which shows a siphoviral morphotype, a plaque size of 5.0 ± 0.3 mm with an expanding halo (7.0 ± 0.3 mm) on extended incubation and genome size of 55,105 base pairs. *Ritam007* shows stability over a wide range of pH (6–12) and temperature (-20°C-55°C) and can infect *M.smegmatis* in stationary phase, under hypoxic conditions and to its isoniazid-resistant strain as well. The presence of halo and stable antimycobacterial activity of *Ritam007* under diverse conditions insinuates its use against mycobacterial biofilms, which play a major role in antibiotic resistance. We found *Ritam007* to inhibit both *M.smegmatis* colony biofilm (qualitatively) and biofilms at the liquid-air interface with an inhibitory percentage of 52.71 % at 10^4 pfu/ml, as quantified by Crystal Violet assay. Phage genome annotation followed by mass spectrometry indicated Gp 6-8, Gp12, Gp15, Gp18, Gp22, Gp24 and Gp25 as the structural genes. Putative identification of functional genes included genes for DNA encapsidation, lysis, replication/transcription, integrase among others and revealed the unique presence of an additional putative LysinA (Gp32) and Holin (Gp40) besides the lysis cassette of LysinA (Gp34), LysinB (Gp35) and Holin (Gp37). To analyse the predicted lysins, we have purified and assayed the activity of the LysinA (Gp34) from the cassette and a comparative analysis with the additional LysinA (Gp32) is underway. LysinA (Gp34) has an amidase and a peptidoglycan recognition (PGRP) domain with an overall molecular weight of 29 kDa. The protein was overexpressed in *E.coli* BL21(DE3) and purified using Ni-NTA+ chromatography. Purified recombinant LysinA (Gp34) was found to have catalytic activity comparable to lysozyme from chicken egg white when tested for their muramidase activity against fluorophore labelled *Micrococcus lysodeikticus* cell walls. Considering the physiological properties, unique lysis cassette and antibiofilm activity, *Ritam007* appears to be a promising candidate for molecular studies and as a therapeutic agent. Therefore, studying this phage can further our understanding of F4 sub-cluster phages (only 4 are reported so far) and of the limited repertoire of mycobacteriophage-derived LysinA and also provide useful therapeutic solutions against mycobacterial infections and the AMR conundrum.

Keywords: Mycobacteriophage; Genome Annotation; Biofilm; Lysin; Lysozyme assay

[Poster 12]: Combining a Phage/Arbuscular Mycorrhizal Fungi-based protective formulation against the plant pathogen Xanthomonas hortorum pv. Vitians

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Lettuce (*Lactuca sativa* L.) is one of the most popular vegetables worldwide, and bacterial leaf spot (BLS) and headrot caused by *Xanthomonas hortorum* pv. *vitians* (*Xhv*) places a major constraint on lettuce production. Moreover, *Xhv* has become a preoccupying threat for lettuce producers over the last decades in the absence of efficient means to control the disease. Currently, copper sulphate-based treatments (Bordeaux mixture) can be used for *Xhv* early prevention. Unfortunately, the application of copper compounds and other agrochemicals has a strong environmental impact, and is often related to the appearance of bacterial resistance/tolerance.

The major challenges associated to control plant pathogenic bacteria stem from the fact that plant production is carried out in an extremely hostile environment, presenting biotic and abiotic factors that may impair phage persistence. In order to maximize phage effectiveness on crop productions, phage quality and stability, association with protective microorganisms has been proposed. The aim of this project is to design innovative root inoculum for lettuce plants, combining the bio-fertilizing properties of arbuscular mycorrhizal fungi (AMF) and bio-protective of bacteriophages specific to the phytopathogenic bacterium *Xhv*.

In our studies, we isolated four bacteriophage active on *Xhv* and we selected *Xhv*-Phage4, the best virulent candidate to further characterization. We demonstrate that *Xhv*-Phage4 is stable at 20°C for at least 5 weeks on *in vitro* conditions. In addition, AMF/*Xhv*-Phage4 association does not inhibit phage stability and protects the phage against UV inactivation after 5 weeks of UV-exposure.

The implementation of a AMF/phage-based association on *in planta* conditions is ongoing. The reported AMF/phage association represent an attractive product for the biocontrol against plant pathogenic bacteria. Moreover, This product will be compatible with Organic and Conventional Agriculture

The present study is supported by Montpellier University of Excellence (MUSE) project.

Keywords: Phage-Based Biocontrol; Plant Pathogen; Xanthomonas hortorum pv. vitians; Formulation

[Poster 13]: Progress in interferometric microscopy

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We previously described a set up which is able to count phages and determine their diameter from 30-35nm in diameter to 100nm and more by following their Brownian motion (diffusion). In addition we showed that we could differentiate viruses from membrane vesicles based on their different refractive index (a).
Progresses have been directed towards a noticeable gain in sensitivity and we are now able to differentiate full and empty capsids (T5 phage) as well as to detect virus of smaller sizes (25 nm MS2 phage).
Moreover our experimental approach has led to explore new fields:
We were interested to quantify metabolic activity within cells in different environmental conditions (b).
To study viral factories within bacteria we analysed artificial aggregates of phages or capsids. We also studied the dynamic of biomolecular condensates implying RNA molecules using a simplified model. We were able to monitor dynamic signals at the edges of these condensates independently from their Brownian motion.
We could also described complex communities of microorganisms such as kefir grain suggesting that this observation could be used to guide genomic analysis of biofilms composition for example or study phage-biofilm interactions.
All these results would be presented and discussed.

Keywords: phage detection; viral factories; community

[Poster 14]: Evaluation of a phage cocktail to prevent avian colibacillosis

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Avian pathogenic *Escherichia coli* (APEC) strains are responsible for the main bacterial disease in poultry, namely colibacillosis [1]. Treatment of this disease relies mainly on the use of antibiotics, which eventually led to the emergence of antibiotic resistant strains. Therapeutic alternatives are needed, and phage therapy represents a promising alternative [2].
Our objective was to evaluate the efficacy of a phage cocktail to prevent colibacillosis induced by the APEC strain BEN4358 in chicks. We have isolated and sequenced 19 different phages (belonging to 9 genus) active against *E. coli*, and for some of them, also active against *Salmonella*. A cocktail constituted of 4 phages of different genus (*Phapecoctavirus, Nonagvirus, Mosigvirus* and *Tequatrovirus*) active against BEN4358 was tested.
In a chicken embryo lethality assay [3], we observed that the phage cocktail allowed 90% of chicken embryos to survive an infection by BEN4358, in contrast to the control (BEN4358 only), which gave a survival rate of 0%.
Then, the efficacy of the phage cocktail to prevent colibacillosis induced in chicks was evaluated. Embryonated chicken eggs were inoculated by the phage cocktail in the allantoic fluid at 16 days of embryogenesis. At day 19, embryonated eggs were transferred from the egg incubator to isolators at day 19, where the chicks hatched at day 21. The APEC strain BEN4358 was inoculated subcutaneously at one-day-old chicks. Mortality was monitored during 7 days. Bacterial and phage charge were determined in liver and caeca of the surviving animals after euthanasia. When phages were detected, they were identified by PCR. Phage susceptibility of the recovered bacterial clones was determined. There was no difference in the mortality rate between both groups, however the mortality was delayed in the group that received the phage cocktail *in ovo*. Moreover, the intestinal BEN4358 load was statistically significantly lower in the phage-treated group (2.2x107 CFU/mL) compared to the non-treated group (1.3x108 CFU/mL).
ESCO3 was detected in the caeca in all (100%) of the surviving chicks, and REC in most of them (92%), highlighting their ability to persist and multiply *in vivo*. 92% of bacterial clones isolated from caeca were resistant to ESCO3, but only one (8%) was resistant to REC. Moreover, the majority of the resistant clones were less virulent in a chicken embryo lethality assay.
The action of phage cocktail inoculated *in ovo* allowed to decrease the intestinal carriage and the bacterial virulence, thus a reduction of the contamination by the environment.

Keywords: avian pathogenic Escherichia coli; phages; cocktail; chick; disease; therapy

[Poster 15]: Oxidative stress alters the interactions occurring in a community of intestinal bacteria and bacteriophages

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Reactive oxygen species (ROS) play a central role in the pathogenesis of inflammatory bowel diseases (IBD). These diseases are characterized by altered abundance and diversity of both bacteria and bacteriophages (phages) of the intestinal microbiota but the mechanisms underlying these changes in composition during chronic inflammation remain largely unknown. This study aims to identify the impact of ROS-induced oxidative stress on the interactions between intestinal phages and bacteria.
We set up a reductionist *in vitro* model using a defined microbial community of three *Escherichia coli* strains and a virulent phage growing in continuous culture in bioreactors. We compared the evolution of this community in the presence and absence of oxidative stress, by exposing the culture to continuous addition of hydrogen peroxide.
We studied population dynamics and the profiles of resistance and infectivity and linked these profiles to the frequencies of genomic mutations of the four populations isolated at different time points over 10 days. While hydrogen peroxide did not significantly alter bacterial viability and phage infectivity, phage concentrations were significantly lower during co-culture under oxidative stress, supporting observations of decreased virulent phages in IBD. Also, our phage populations evolved to be more specialist to their isolation strain in the presence of oxidative stress compared to the control condition. Finally, genomic analysis highlighted high-frequency mutations, signatures of phage-bacteria arms-race and a comparative analysis is currently ongoing.
These data contribute to the fundamental understanding of how environmental variations may affect bacteria-phage interactions and hence the equilibrium of the gut microbiota. Determining the impact of inflammation-driven abiotic factors in altering microbial diversity is a step towards understanding the pathophysiology of IBD.

Keywords: coevolution; gut microbiota; oxidative stress; resistance

[Poster 16]: Systematic and quantitative view of the antiviral arsenal of prokaryotes

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Bacteria and archaea have developed multiple antiviral mechanisms. Since 2015, dozens of new systems have been discovered. Genomic evidence indicates that several of these antiviral systems co-occur in the same strain. Here, we introduce DefenseFinder, a tool that automatically detects known antiviral systems in prokaryotic genomes. Following the recent update, DefenseFinder can detect more than 100 defense systems and 200 defense system subtypes. Here we present the analysis of more than 21000 fully sequenced prokaryotic genomes using DefenseFinder. We find that antiviral strategies vary drastically between phyla, species and strains. Variations in composition of antiviral systems correlate with genome size, viral threat, and lifestyle traits. DefenseFinder will facilitate large-scale genomic analysis of antiviral defense systems and the study of host-virus interactions in prokaryotes.

Keywords: Defense system; bacterial immunity; phage

[Poster 17]: Biocéramiques et Phagothérapie

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Depuis plusieurs décennies, les céramiques à base de phosphate de calcium (hydroxyapatite et phosphate tricalcique β) ont été utilisées comme substituts afin de remplacer les défauts osseux en chirurgie ostéo-articulaire du fait de leur composition minérale similaire à celle de l'os et de leur très bonne biocompatibilité vis-à-vis des cellules ostéoblastiques. Actuellement, deux problèmes limitent l'efficacité des greffes de prothèse ou de substituts osseux en chirurgie ostéo-articulaire : la contamination au cours de l'opération par des bactéries pathogènes et le défaut d'ostéogénèse complète au sein de ces substituts. Après une infection ostéo-articulaire, certains patients traités par antibiothérapie se retrouvent asymptomatiques mais peuvent malheureusement subir une récidive de la même infection d'origine. Cette récidive s'explique par la présence de biofilm bactérien sur les prothèses ou substituts osseux agissant dans l'organisme comme une maladie silencieuse. Pour cela, nous nous proposons d'étudier l'efficacité des traitements par phagothérapie sur un biofilm bactérien mature de plusieurs jours fixés sur céramiques à base de phosphate de calcium ou sur d'autres supports dans l'objectif d'utiliser les bactériophages en curatif. L'efficacité de l'utilisation de dispositifs céramiques à base de phosphate de calcium chargés en bactériophages a déjà été prouvée au sein du laboratoire sur des cellules planctoniques et biofilms mature d'une journée. L'objectif de notre étude est d'améliorer ces dispositifs afin d'augmenter d'une part le relargage continu de bactériophages au site infectieux mais également de faciliter la diffusion des phages au sein du biofilm. En effet, certains bactériophages ne diffusent pas naturellement à travers la capsule des biofilms bactériens.

Keywords: Biomatériaux; Phagothérapie; Biofilm; Staphylococcus aureus

[Poster 18]: Translocation of bacteriophages across the intestinal barrier: relationships with paracellular permeability and inflammation

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Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, are chronic and progressive disorders that can lead to bowel damage. It is now well recognized that the intestinal microbiota composition is an important factor in the pathogenesis of IBD. Among the IBD-related dysbiosis, alterations in bacteriophage (phage) abundance and diversity have been highlighted in patients but their contribution to these pathologies remains poorly understood. Previous results from the laboratory showed that faecal phages were detected at a higher rate in the blood of IBD patients compared to healthy subjects. We hypothesised that the rise in gut permeability in IBD might result in higher phage translocation. By combining *in vitro* and *ex vivo* studies, we aimed to decipher the interaction of phages with the intestinal barrier under basal and inflammatory conditions. T4 or M13 phages were applied to the apical side of Caco-2/TC7 intestinal epithelial cells cultured on transwell filters or to the luminal compartment of mouse gut samples mounted in Ussing chambers. Phages from the basal side were quantified at different timepoints. EGTA or pro-inflammatory cytokines, TNF-α and IFN-γ, were used to increase the paracellular permeability of epithelial cell monolayer. As expected, epithelial paracellular permeability was significantly higher in the presence of EGTA or inflammatory cytokines. Our results showed that inflammatory conditions increase the translocation of T4 phages across the epithelial cell monolayer after 24h of incubation. However, T4 phages did not induce a hyperpermeability either in physiological or in inflammatory conditions. Moreover, T4 phages did not increase the release of lactate dehydrogenase nor the secretion of inflammatory cytokine IL-8 in the basal medium after 24h of incubation. Also, translocation of M13 and T4 phages from the luminal to the basal side was observed in mouse ileon and colon tissues mounted in Ussing chambers, where T4 phages translocate at higher rates than M13. Collectively, these first data demonstrate that under inflammatory conditions, phages are able to cross the epithelial barrier at higher rate without triggering intestinal permeability, cytotoxicity and inflammation. Additional investigations including other phages and cell types are ongoing. Such study is an important step to better understand phage-human cells interactions in patients with IBD.

Keywords: intestinal microbiota; bacteriophages; paracellular permeability; inflammation; IBD

[Poster 19]: "Jack of all strains, master of all"? Phylogenetic host range of phages correlates with phage virulence

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Bacteriophages (phages) hold the promise of being the perfect hunters of pathogenic bacteria in a wide range of applications, from medicine to industry or agriculture. As a consequence of the extreme specificity of most phages, a broad host range is the main criterion of phage selection in therapeutic cocktails. Considering evolutionary trade-offs among phage characteristics such as phage efficacy and host range, could refine the selection of candidate phages and will help the durability of many phage applications.
Bacterial wilt caused by the *Ralstonia solanacearum* species complex (RSSC) is among the most important plant diseases worldwide, severely affecting a high number of crops. Our aim was to isolate and study new phages capable of infecting the RSSC, to be used as biocontrol tools in the South West Indian Ocean. A high genetic diversity was found in 23 phages infecting RSSC isolated in Mauritius and Reunion islands, with 7 new genera and 13 new species. In an innovative approach to assess the host range of phages, phylogenetic data of the targeted bacteria was integrated in the analysis, creating a phage phylogenetic host range index (PHI). We demonstrate that all phages preferentially attack the most abundant RSSC variant in both islands, but harbour a high host range variability. The 23 phages also show differences in their virulence, i.e. their efficiency at decreasing bacterial growth *in vitro*. We demonstrate a positive correlation among the phage PHI and their virulence for Mauritian phages, but no pattern for Reunion ones. Our results point out that phages with a wide host range are optimal candidates for biocontrol in complex epidemiological situations, such as in Mauritius island, but that this pattern is not extensive to every phage-bacteria interaction. We also show that different phages from the same species are able to target very different RSSC strains, suggesting that phage genomes and host range can rapidly evolve.

Keywords: host range; evolutionary trade-offs; biocontrol

[Poster 20]: Identification of the receptors of four therapeutic phages used in human phage therapy

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Pseudomonas aeruginosa is a ubiquitous Gram-negative bacterium found in numerous freshwater and terrestrial environments. It is also an opportunistic pathogen that causes various infections in humans such as airways infections in cystic fibrosis patients and pneumoniae in patients on assisted ventilation as well as bloodstream, urinary tract, burn wounds infections. Moreover, *P. aeruginosa* is one of the six pathogenic bacterial species of the ESKAPE group, formed of multi-drug resistant bacteria, which infections are getting harder to clear, with fewer antibacterial drugs still efficient against them, resulting in higher mortality and morbidity rate. For this reason, novel therapeutic strategies beside antibiotics must be developed. Phage therapy is one promising alternative strategy.
Phage resistance emergence is a phenomenon that clinicians, researchers, and regulatory agencies want to minimize. Unfortunately, the phage-bacteria coevolution is continuous and responds to the selection pressure that each partner exerts on the other. One of the resistance causes is the modification of the phage specific receptor on the bacterial surface. To determine the receptors of our phages, the WT strain was incubated in the presence of phages. Mutant strains with mutation either in *wzy* or *pslD* were selected as respectively resistant to two myoviruses, PP1450 and PP1777 or to two podoviruses, PP1792 and PP1797. The activity of four phages was studied on three isogenic strains of *P. aeruginosa*, the WT strain and the *wzy** and *pslD** mutants, was studied by measuring their ability to form lysis plaques, their capacity to inhibit bacterial growth and their adsorption rate. The results show on the one hand, that the mutation of *pslD* leads to the inhibition of the podoviruses phages efficiency, in each test: no more ability to form lysis plaques, to inhibit bacterial growth and to adsorb on the bacterial surface. On the other hand, the mutation of *wzy* leads to the inhibition of phage activity, in each test, for the myoviruses. It thus demonstrates that the psl exopolysaccharides exported by PslD is the receptor of the two podoviruses, genetically related to the Luz24 phage, whose receptor was still unknown; and second that the O chain of lipopolysaccharides, polymerized by Wzy, is the receptor of both myoviruses, as expected as these phages are related to Ab27.
Finally, the combination of phages of the two families, having different receptors, decreases the frequency of resistance appearance. These results are a crucial step in understanding bacterial phage resistance.

Keywords: phage receptor; pseudomonas aeruginosa; phage resistance

[Poster 21]: Fishing for phage lineages to understand their evolution

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Vibrio crassostreae is a bacterial pathogen associated with the Pacific Oyster Mortality Syndrome. We previously showed that this population is highly dynamic and we hypothesized that predation by virulent phages affects *V. crassostreae* infection dynamics on two levels. First, phages reduce pathogen density and second, they select for less virulent but phage-resistant vibrios. Investigation of the genetic mechanisms driving phage specificity identified a two-step host protection, first involving receptor recognition, then intracellular host defense systems. The adsorption pattern of specific phage genus matched near-clonal bacterial clades within the *V. crassostreae* species. Presence of intracellular defense systems further narrowed the range of strains that could be successfully infected and killed.
Here we decided to focus on this genetic unit (the vibrio clade and the phage genus) to further explore ecological and evolutionary processes underlying bacteria and phage dynamics in natural systems. We performed a large sampling of phages and vibrios in an oyster farm (summer 2021) to identify co-evolving lineages and understand the genomic and mechanistic foundations of phage–vibrio interactions. We generated archives of virus concentrates (from both sea-water and oyster plasma), total community DNA (4 seawater size fractions and oyster hemolymph), metaviromic DNA, total community glycerols preserving live cells, and environmental metadata during the time-series lasting. Roughly 500 *V. crassostreae* isolates were obtained throughout the beginning, middle, and end of the time-series. Initial investigation revealed that most of the isolates could be grouped with previously described clades, suggesting clonal expansions. Additionally, we used a previous collection of 157 *V. crassostreae* strains (with several representatives of each clade) to estimate strain-level viral predation rate and to isolate 1200 phages. Genotyping revealed that a large proportion of them belong to previously identified phage genus, demonstrating multi-year stability of both vibrio clades and phage genus. Finally, we investigated the abundance of phage genus and vibrio clade over time and space to support the key role of phages in controlling this oyster bacterial pathogen.

Keywords: phage/vibrio ecoevolutionary dynamic

[Poster 22]: Mismatch repair and phage mutation rates

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Compared to their cellular hosts, viruses have much higher mutation rates, even DNA viruses that use their host's replication machinery. Mutation rate estimates for double-strand DNA phages are close to 10–8 mutations/nucleotide/replication (m/n/r) in lambda and T4 phages, while the mutation rate of their *E. coli* host is close to 10-10 m/n/r. in *E. coli,* the highly conserved MMR post replicative repair system detects DNA replication errors and repairs up to 99% of them.
We show that MMR system is inefficient on lambda phage, as was previously shown on T4 phage (Santos & Drake, 1994), which could explain the high phage mutation rates. A first explanation of this inefficiency could be that replication errors on phage DNA are not accessible to MMR proteins or well recognizable by them. Using fluorescently tagged MMR protein MutL that allows visualizing DNA mismatches as bright fluorescent foci inside the cells, we show that the MMR system detects replication errors on lambda DNA but not on T4 DNA. We are now investigating the reasons of this phenomenon on both phages.

Keywords: mutation rate; replication errors; lambda; T4; microscopy

[Poster 23]: Host DNA destruction and the (subtle?) puzzle of phage T5 pre-early genes

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The virulent bacteriophage T5, which infects *Escherichia coli*, injects its 122-kb linear dsDNA in two steps: 8% of the genome (carrying 17 so-called pre-early genes) are first transferred into the cell and the injection pauses. Following expression of these viral genes, the host DNA is degraded and cellular defense systems inactivated. Viral DNA transfer resumes after 3-5 minutes to allow entry of the rest of the genome that encodes early and late proteins necessary for viral DNA replication, virion assembly and release. So far, mutants in only three pre-early genes have been characterized: (i) *dmp* encodes a 5'-deoxynucleotidase that is dispensable for infection, (ii) gene *A2* encodes a putative DNA-binding protein that is essential for the viral DNA second step transfer (SST) and (iii) gene *A1* encodes a predicted nuclease that is required for host DNA digest and SST. Thus, T5 constitutes an original model to study host takeover and uncover viral effectors targeting host cell functions. In this study we investigated the role of the remaining pre-early genes in T5 infection kinetics and host DNA degradation. Genetic engineering strategies were successfully developed to introduce amber mutations, as well as single and multiple gene deletions in T5. Besides *A1* and *A2*, none of the 13 genes analyzed were essential for T5 infection in laboratory conditions. Several mutants exhibited changes in latent period, burst size and virulence, suggesting that at least 2 other pre-early genes facilitate viral infection. Fluorescence microscopy analysis revealed that only *A1* was required for host DNA destruction, but not the other two predicted nucleases. Taken together, our study uncovered critical T5 pre-early genes needed for host takeover.

Keywords: deoxyribonuclease; host takeover; Bacteriophage T5

[Poster 24]: Safety and efficacy of an AIEC-targeted bacteriophage cocktail in a mice colitis model

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Adherent invasive *E. coli* (AIEC) are recovered with a high frequency from the gut mucosa of Crohn's disease patients and are believed to contribute to the dysbiosis and pathogenesis of inflammatory bowel disease. Thus, targeted removal or reduction in the colonization levels of AIEC in IBD patients may have important clinical implications. In this context, bacteriophage therapy represents an intriguing approach for specifically targeting AIEC in the human gut with no deleterious impact on the commensal microbiota. In this study, we report the *in vitro* efficacy and specificity of a seven lytic phage cocktail (EcoActive™) against a (i) large number of clinical AIEC strains, and (ii) a panel of non-*E. coli* strains representing the top 12 most common genera typically associated with a healthy human microbiome. Long-term administration of phage cocktail to healthy mice was safe and did not induce dysbiosis according to metagenomic data. We further describe the impact of this phage cocktail on DSS-induced colitis of mice infected with the AIEC strain LF82. Whereas a single administration failed to alleviate inflammatory symptoms, mice receiving the cocktail twice a day for 15 days were protected from clinical and microscopical manifestations of inflammation. Collectively, the data suggest that AIEC-targeted phage therapy may be a safe and effective approach for reducing AIEC levels in the gut and for treatment of IBD patients.

Keywords: bacteriophages; AIEC; colitis; IBD; Crohn's disease

[Poster 25]: Phage T5 encodes a new pathway for RpoS degradation, the master regulator of the general stress response in E. coli

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Host takeover by bacteriophages is a prerequisite for successful infection. In phage T5, it is uncoupled from the rest of the lytic cycle, thanks to T5 unique ability to transfer its genome in 2 steps [1]. During the First-Step Transfer (FST), pre-early genes (about 8% of T5 DNA) are injected, which are sufficient to degrade host DNA, evade cellular defenses and hijack the cellular machineries. Both the FST-encoded A1 nuclease and A2 DNA-binding proteins are involved in host takeover, and required for resuming DNA transfer. In E. coli, the sigma factor RpoS is the master regulator of the general stress response (GSR). Under optimal growth conditions, RpoS interacts with the adaptor RssB and is then degraded by the ClpXP protease: the GSR is thus silenced. Under stressful conditions, anti-adaptor proteins prevent RssB/RpoS interaction, leading to RpoS accumulation, which can then fire the GSR [2].
Phages evolved various strategies to temper with RpoS action, for example by preventing its binding to promoters as is the case with phage T7 Gp5.7 protein directly interacting with RpoS [3]. Here we show in T5 that RpoS degradation via ClpXP is triggered upon infection during FST, independently from A1 and A2 but surprisingly also from RssB, the only known RpoS adaptor to date. The aim of our project is to investigate the FST gene product(s) responsible for this phenotype.
We show here that a FST fragment only containing gp016 and its 100 bp-upstream regulatory region is sufficient to trigger RpoS degradation, suggesting a new pathway to target RpoS to degradation by ClpXP, and independently of RssB. Our bioinformatics analyses suggest the presence of a promoter and a putative small ORF of unknown function (ORFX) upstream gp016. We present here our investigations on the genetic elements encompassed in this FST small fragment required for RpoS degradation by ClpXP independently from RssB.
This work highlights a novel strategy evolved by a phage to temper with the host GSR.

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Keywords: Interactions; Defense strategy; Phage T5; RpoS Master regulator

[Poster 26]: Isolation and characterisation of virulent phages against European Xylella fastidiosa subspecies

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Xylella fastidiosa (Xf) is ranked in the world top 10 most important bacterial plant pathogens [1] and its host range is now estimated at 638 plant species [2]. This bacterium colonizes the xylem vessels of plants and forms biofilms that block water flow, resulting in plant wilting and eventually death [3]. *Xf* is transmitted by biting-sucking insects.
The most spread and damaging subspecies (*Xf fastidiosa*, *Xf pauca*, and *Xf multiplex*) are present in many countries [4]. Therefore, *Xf* is listed as a quarantine pest in Europe [3,4] and its potential economic, environmental and social impact are considered the most serious in the Union [5]. Since 2013, *Xfp* has been identified on olive trees in Apulia in Italy [6]. In Spain, the situation quickly deteriorated on olive and almond trees, and in France heavy infection have been reported in the PACA region. Since 2017, Corsica is entirely infected by *Xfm* strain.
No curative or prophylactic technique exists against *Xf*. It is thus of global importance to find sustainable means of biocontrol. Phages are promising and our objective is to develop a cocktail targeting Mediterranean strains. For this, *Xf*-associated and non-*Xf*-associated environments were tested to find phages.
To bypass the difficulties of *in vitro* experimentation on *Xf*, a surrogate host strategy on *Xanthomonas albilineans* was implemented. After three years of research, three phages were selected as candidates [7]. These results led to further research, isolation and characterization of phages. To date, we have about twenty characterized phages having at least efficiency on *Xfm*, some being also efficient on *Xff*.

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Keywords: phage therapy; biocontrole

[Poster 27]: Quantification of virus driven plaque expansion in real time

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Almost every biology book describing bacteriophages will invariably include a picture of a petri dish covered by lysis plaques. These round-ish clearings in a bacterial lawn are the result of a complex dynamic process of phage replication within an immobile host population. Plaque formation involves phage outward diffusion from an initial spot, and its expansion is fed by new infection events of the cells situated at the edges of the expanding plaque. Multiple factors have an impact on phage propagation and several models have been proposed to characterize it. However, a detailed recording of phage propagation in (semi)solid medium has never been performed due to the technical challenges imposed by the structural complexity of a plaque. In this study, we aim to understand how antibiotics, in sublethal concentrations, can increase the speed of plaque growth, in a phenomenon known as Phage-Antibiotic Synergy. To achieve this, we introduce a multiscale (macro and microscopic) real-time tracking of the expansion of a lysis plaque of coliphage T7 in an *E. coli* lawn. This allowed us to observe and quantify the development of plaque formation with unprecedented detail in the presence of two synergistic antibiotics: Ciprofloxacin and Mecillinam. Although both increase plaque size, their effects on the cell morphology are very different, the first induce bacterial filamentation, and the second produce short, round-shaped cells.
Using a binocular loupe, we recorded plaque formation and observed that mecilinam produces the largest increase in phage propagation speed. This was reproduced in *E. coli* Δ*fabH* consisting of abnormally small cells. This suggests a strong morphology-driven effect on phage propagation. We hypothesized that smaller cell surfaces imply less adsorption, which allow virions to diffuse further.
Secondly, to study why filamentation also produces enlarged plaques we developed a pioneering method to study the epidemic spread of phage at microscopic resolution. The study of phage infection was improved by the introduction of a parS cassette in the T7 genome that allowed to observe the apparition of foci upon T7 dsDNA injection in ParB-GFP coding *E. coli*. Thanks to this, we observed a phenomenon that can account for faster phage spread: antibiotics delay cell division, reducing host density. However the presence of multiple foci appearing in those filaments suggests that phage propagation is not disrupted by the halt on cell division. This gives the phage an unexpected advantage to propagate in a more efficient pattern despite the presence of the drug.

Keywords: Phage; Antibiotic; Synergy; Microscopy

[Poster 28]: Phages against non-capsulated Klebsiella pneumoniae: broader host range, slower resistance

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Background: Phage efficiency relies on their ability to recognize and bind on the bacterial surface, which depends on high specificity towards surface structures. This makes *Klebsiella pneumoniae* (Kp) phages highly specific towards its most external component, the polysaccharidic capsule. However, resistance to capsule-targeting phages have been shown to evolve rapidly, leading to the exposure of other membrane structures. In this work, we devised a strategy to isolate phages that target capsular mutants. These phages may be useful in phage cocktails as they would target resistant mutants emerging during treatment with first-line phages. We also aim to characterize the phage-*Klebsiella* host interactions.
Methods: Based on a collection of 7,388 *Klebsiella* genomes, we selected six wild type Kp and seven capsular mutant strains for their surface structures prevalence and diversity. Phages were isolated and genomically characterised. Phenotypic characterisation including host-range, infection curves and efficiency of plating was also performed. Phage resistant Kp clones were isolated and sequenced. Phage efficiency experiments in vivo were performed using OMM12 mice model.
Results: 68 phages, 27 phages using 7 capsule-deficient Kp strains as hosts (anti-Kd phages), and 41 phages against 7 wild-type (wt) Kp strains (anti-K phages) were isolated belonging to 4 different taxonomic families. Anti-Kd phages have a broader host-range and resistance against them took longer to emerge in vitro. We also discovered one broad host range phage that infected virtually all different O-antigen types of Kp. Resistance to these phages evolved through mutations in O-antigen or LPS synthesis genes. We could observe in vivo replication of both phage-types, targeting or not the capsule.
Conclusion: This study demonstrates the feasibility of a new strategy to isolate phages against non-capsular structures and that have a broad host-range within Kp. Such phages may represent interesting additions for phage therapy cocktails.

Keywords: Klebsiella pneumoniae; resistance; host range; in vivo

[Poster 29]: PHAGEinLYON - PHAG-ONE: Isolation and characterization of phages active against multidrug-resistant ST131 E. coli strains producing carbapenemases and/or extended-spectrum beta-lactamases

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Introduction and objectives
Antibiotic resistance is a major public health issue. The global spread of *Escherichia coli* strains belonging to the sequence type (ST) 131 is of particular concern, since a high proportion of them produced extended-spectrum beta-lactamases (ESBLs) and/or carbapenemases (CPs). These enzymes confer a multidrug resistance profile that results in infection for which no further therapeutic options are currently available. Phage therapy is one of the alternatives to treat infection and/or eradicate colonization. In this context, the aim of the present study was to identify anti-*E. coli* ST131 phages from the environment and to evaluate their activity on a panel of 191 clinical isolates representative of this ST and producing CPs and/or ESBLs.

Materials and methods
Phages were isolated from sewage after liquid enrichment using an initial panel of 12 *E. coli* strains representative of the ST131 genetic diversity, and selection on double-layered plates. The activity profile of phages was determined by plaque assay on ST131 clinical strains (n=191) and their genome sequenced using Illumina technique. Phage activity based on Efficiency Of Plating (EOP) ratios was analyzed according to ST131 sub-clades, CPs/ESBLs types and phage genera.

Results, discussion and conclusion
Twelve phages were isolated. Their profiles of activity varied from 5 (3%) to 164 (86%) strains out of the 191 clinical strains of the panel tested. The three phages with the broadest spectrum belonged to the *Tequatrovirus* genus, and respectively exhibited activity on 86% (n=164), 81% (n=155) and 71% (n=136) of the strains. Phage activity was not significantly related to ST131 sub-clades and CPs/ESBLs contents. Interestingly, two out of the twelve phages, belonging to two different genera (*Tequatrovirus* and *Vectrevirus*), showed the largest complementary activity, enabling to target 93% (n=177) of the clinical strains of the panel. Of note, eight strains were resistant to all of the twelve phages. The analysis of phage activity in liquid media, alone or in combination, is underway and will be presented.
Here, we report the isolation and characterization of strictly lytic phages active against a wide selection of multidrug-resistant ST131 clinical *E. coli* strains. Some of these phages are good candidates for phage therapy and have been included in the PHAG-ONE pipeline for pharmaceutical production and *in vivo* activity testing using animal models. The GWAS approach and the search for anti-phage defense systems are ongoing to identify the genetic determinants of phage activity.

Keywords: Bacteriophages; Phage therapy; Escherichia coli ST131; Carbapenemases; Extended spectrum beta lactamases

[Poster 30]: Directed in vitro evolution of anti-Staphylococcus aureus therapeutic phages: host range expansion against multi-drug resistant Staphylococcus epidermidis isolates

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Introduction
Bacteriophages are a promising strategy to tackle multidrug resistance and to improve the outcome of biofilm-associated infections, such as staphylococcal bone and joint infections. Contrary to *Staphylococcus aureus*, very few phages active against *S. epidermidis* (SE) have been described, while it is a major pathogen in this context. Phage training, consisting in forced adaptation of phages against initially resistant bacteria, could be an alternative to classical phage discovery based on isolation of anti-SE phages from environmental samples, which is fastidious and frequently inconclusive.
Methods
According to the "Appelmans protocol", we cultured for 30 rounds a mixture of three previously characterized *Herelleviridae-Kayvirus* anti-*S. aureus* phages (V1SA9, V1SA12 and V1SA15) on a panel of bacterial isolates including 6 phage-resistant SE isolates. Adapted phage subpopulations were isolated from this "trained mixture" on bacterial strains for which a gain of activity was observed (5 Plaque-Forming-Units/strain). Host range modifications of adapted phages were evaluated using a large panel of *S. epidermidis* (n=68) clinical isolates belonging to 11 sequence types (ST), *S. aureus* (n=30) and other coagulase negative staphylococci (n=35) isolates. A Genome-Wide-Association Study (GWAS) was performed to identify bacterial determinants of susceptibility to trained phages. Genomic modifications associated with phage training were assessed comparing genomes of adapted and ancestral phages.
Results
The trained phage mixture was active against 2/6 SE resistant strains, named A (ST2) and B (ST35). In addition, the trained subpopulations A-1 to A-5 were only active on some of the ST2 SE strains (17/47) while subpopulations B-1 to B-5 were active only on their isolation strain. Of note, adaptation to SE was associated to an significant loss of activity against other staphylococcal species.
GWAS results showed that the presence in bacterial genomes of a gene encoding an uracil-DNA glycosylase inhibitor surrounded by recombinase genes was associated to resistance to trained phages among ST2 strains.
Finally, adapted phage genomes originated from multiple recombinations between genomes of ancestral phages V1SA9 and V1SA15 that were located in two hotspots corresponding to replication and tail proteins coding genes. Further studies are underway to explore their impact in host range modifications.
Conclusion
We report for the first time that phage training can be used to enlarge phage host spectrum from a bacterial species to another. Adaptation to SE was however limited to one ST and was associated to specialisation to this bacterial species.
Keywords: Phage training evolution; staphylococcus; host range

[Poster 31]: Impact of faeces preparation methods for the study of the virome by NGS

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Viruses constitute an important part of the faecal microbiota. The differences in structure and size may impact the efficiency of the methods used to purify, concentrate and amplify their genome.

The aim of the study was to combine different processes to purify and concentrate virions from faeces, and to analyze the impact on their identification by Next-Generation Sequencing (NGS).

We studied 6 faecal samples from 5 healthy volunteers. Seven techniques were followed. Six were defined according to the methods of purification and concentration of the virions used combining various A) purification methods : dilution in PBS ± DTT followed by clarification or use of the Stool Preprocessing Device® (SPD®, Biomérieux®), then filtration (0.45 µm), and B) concentration methods of the virions : precipitation using the PEG Virus kit Precipitation Kit® (CliniSiences) or centrifugation on Centriprep® (Sigma-Aldrich®). All the samples then underwent the following steps: dialysis, action of a DNase/RNase cocktail (Lucigen, Sigma-Aldrich®), extraction of the encapsidated viral nucleic acids (Macherey-Nagel®), reverse transcription of the genomic viral RNAs into DNAs (Superscript IV®, ThermoFisher Scientific®), double-stranded DNA quantitation (Qubit®, ThermoFisher Scientific®), library preparation (Accel NGS 1S Library Kit® (Swift BioSciences), sequencing on NovaSeq 6000® (Illumina®) with a depth of 2x136 million reads per sample (Institut du Cerveau et de la Moelle épinière sequencing platform at Pitié-Salpêtrière Hospital). The 7th branch was given to the Henri Mondor Hospital sequencing platform and prepared and tested according to routine procedure (MetaMIC® "pan-pathogen" and shotgun sequencing). Bioinformatics analysis of all the FastQ files was carried out by the company Biomanda. The SPP1 phage was used as an internal control at an amount of 10^5 virions per branch.

DNA quantitation results before library preparation and amplification revealed heterogeneity between samples and branches, with higher quantities for SPD®/Centriprep® and PBS/DTT-/Centriprep® branches. After amplification, nucleic acids of all the samples were in sufficient quantity for sequencing and with sizes between 600-750 bp. The branches including SPD® allowed to obtain a higher quantity of DNA. Sequencing results identified phage SPP1 DNA in all branches, except Mondor. For the same sample, viral genomic sequences detected differed according to the branches. The PBS/DTT+/PEG, PBS/DTT+/Centriprep® and PBS/DTT-/Centriprep® branches seemed to detect the greatest number of viral sequences. The PBS/DTT+/Centriprep® and PBS/DTT-/Centriprep® branches seemed to detect the higher diversity in terms of viral families. These results are preliminary and will need to be analyzed in more detail.

Keywords: virus; faeces; purification; concentration; NGS; gut microbiota

[Poster 32]: Characterization of virus-host dynamics in anaerobic digesters under abiotic stress

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Viruses of microbes are major players in various ecosystems, typically affecting the structuration and dynamics of microbial communities. They nevertheless remain poorly explored in anaerobic digestion (AD) plants, where biowaste is valorised into methane-rich biogas. The AD process ecosystems are very sensitive to disturbance - archaeal methanogens are the first impacted - leading to inhibition and loss of methane production. We were therefore interested in better understanding the interplay between abiotic disturbance, microbial community composition, including the viromes, and process performance.
The variations of both viral and prokaryotic populations were followed in batch AD microcosms under abiotic stress. Four types of abiotic disturbances were tested in triplicates. During incubation, either NaCl, NH4Cl or phenol was injected into the reactors, as inhibitors previously reported in full-scale plants. Mitomycin C was also tested, since it can activate the lytic cycle of proviruses. We first confirmed a significant impact of the tested stresses on biogas production. Then we performed 16S rDNA metabarcoding targeting archaea and bacteria, and shotgun metagenomic sequencing of 30 selected metaviromes.
Metavirome coassembly showed a N50 of 3,886 bases, and yielded more than 10^5 contigs longer than 1,000 bp. Among them, 45,914 were predicted as putative viral contigs using VIBRANT (including 2,815 complete). After strict quality filtering, thanks to two more tools, CheckV and VirSorter2, 430 selected contigs emerged as highly reliable. Upon those, 127 were differentially abundant when comparing intra-condition variability before and after disturbance; 16 being common to three inhibitory conditions. SpacePharer was used to decipher some of the host-virus interactions. We will soon be able to interpret the observed community dynamics.
We also detected auxiliary metabolic genes related to carbohydrate metabolism and the sulphur relay system, which seems relevant to this specific ecosystem. Moreover, there was a good agreement between the prokarotyic community composition and the predicted hosts of the viral contigs, with the dominance of Clostridiales, in both cases. Also, we plan to apply the epicPCR technique to confirm specific virus-host pairs.

Keywords: anaerobic digestion; viruses of microbes; microbial ecology; meta-omics; epicPCR

[Poster 33]: Characterization of new molecular partners involved in filamentous phage infection

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Filamentous phages are a subclass of the Inoviridae known to be nonlytic viruses. The persistent association they establish with the infected bacterium can deeply modify the fitness and the pathogenicity of the host. The mechanisms involved during infection are still poorly described, even for the well-known model couple Escherichia coli - phage Fd. The minor capsid protein pIII located at the tip of the phage leads the infection process. This protein is organized into three distinct domains pIII-N1, pIII-N2 and pIII-C. To date, only two interactions between the Fd phage and bacterial proteins have been shown. During an initial stage of reception, the phage Fd binds to the F-pilus thanks to its pIII-N2 domain. Then the phage is translocated across the outer membrane into the periplasm where pIII-N1 interacts with the TolA protein of the Tol-Pal system. The TolQ and TolR inner-membrane proteins also play a role in the infection process while there is no evidence of direct binding with the virus so far. The TolA, TolQ and TolR proteins are part of the Tol-Pal system, a macro-complex of the envelope dependent on the proton-motive force and conserved in Gram- negative bacteria. This system is involved in cell division and in the homeostasis of the cell envelope. The molecular mechanisms subsequent to TolA binding and allowing the phage to open and inject its genetic material across the inner membrane are currently not known. In order to get a better understanding of the late step of filamentous phage infection, we combined different approaches to study protein-protein interactions, such as two-hybrid, co- immunoprecipitation and cysteine scanning. We demonstrated that pIII binds the TolQ and TolR proteins in vivo, and dissected further the subdomains involved in these interactions. Together, our results allow us to propose a new step in the infection mechanism during the translocation of the Fd phage through the host envelope.

Keywords: Filamentous phage; Uptake; Tol Pal system; Virus host protein protein interactions

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