PHAGES in LYON 2023

27-29 nov. 2023
Lyon, Domaine Lyon Saint Joseph
France

Table des matières

Ecologie et Evolution 7

Genomics-based prediction of the phage-bacteria interactions at the species level, Hugo Vaysset [et al.] . . . 8

The diversity and mobilization potential of P4-like phage satellites, Jorge Moura De Sousa [et al.] . . . 9

Origins and rate of mutations in bacteriophage Lambda, Julien Lopez [et al.] . . 10

Study of a potential antiphage system of the "abortive infection" type regulated by a non-coding RNA in Clostridioides difficile, Marion Saunier [et al.] 11

Oxidative stress influences the evolution of a synthetic community of gut bacteria and bacteriophage, Coline Meynard-Doumenc [et al.] . . . 12

Phage susceptibility in phytopathogenic Pseudomonas syringae strains from different ecological niches, Chloé Feltin [et al.] . . . 13

Advances in understanding the ecology of bacteriophages in cheese, Thomas Paillet [et al.] . . . 14

Induction of prophages by bacteriocins in Escherichia coli fecal isolates, Caroline Henrot [et al.] . . . 15

Identifying novel virus families infecting nitrifying archaea and bacteria in soil, Sungeun Lee [et al.] . . . 16

Inferring the life-history traits of new viral variants from epidemiological and evolutionary dynamics, Wakinyan Benhamou [et al.] . . . 17

Bacteriophages in battle – how an anti-phage system distinguishes friend from foe, Jakob T Rostøl . . . 18

Following "Mystery training" *: How a bacteriophage adapts to diverse multidrug-resistant genotypes of E. coli ST131, Elsa Dardalhon Beurrier [et al.] 19

Characterization of virus-host dynamics in anaerobic digesters under abiotic stress, Marion Coves [et al.] 21

Diversité virale des eaux de baignade de la Seine, Alisa Langlais [et al.] 23

Tracking viral infection temporal dynamics after microbial resuscitation during seasonally dry soil rewetting using viromics and stable isotope metagenomics, Ella Sieradzki [et al.] 24

" This Training Is Bound for Glory"* : How a bacteriophage adapts face to diverse bacterial genotypes, Amandine Maurin 25

Spotlight on the viral diversity of fermented vegetables using viral metagenomics, Julia Gendre [et al.] 26

Phage-plasmids produce novel types of plasmids and phages and drive gene flow between them, Pfeifer Eugen [et al.] 28

The virulent Krappator 27 phage selects for unique genomic changes in the lactic acid bacterium Oenococcus oeni., Florencia Oviedo Hernandez [et al.] 29

Structure et Assemblage **30**

Cryo-electron tomography study of bacterial cell wall perforation by bacteriophage T5, D'acapito Alessio [et al.] 31

Structural insights into host-binding machineries of mycobacteriophages revealed by AlphaFold2, Christian Cambillau [et al.] 32

Assembly regulation of pb6, siphophage T5 Major Tail Protein, Charles-Adrien Arnaud [et al.] 33

Mechanisms of assembly and evolution of the dsDNA gatekeeper from the tailed viruses-herpesviruses lineage, Igor Orlov [et al.] 34

Probing the world of phage specificity: a glimpse into receptor binding protein structures and their development as diagnostic bio-probes, Matthew Dunne . . . 35

Interaction Phage-hôte **36**

Characterization of a (p)ppApp synthetase belonging to a new family of polymorphic toxin associated with temperate phages, Julie Viala [et al.] 37

Bacteriophage 933W Can Reach Brain Tissues of Escherichia coli Monocolonized Mice, Provided its stx Eukaryotic Promoter is Functional, Marie-Agnès Petit [et al.] 38

The phages which don't kill Xanthomonas hortorum pv. vitians, doesn't make it stronger: molecular determinants and trade-off in planta, Anaelle Baud [et al.] . . 40

Deciphering the interaction specificity of Clostridioides difficile bacteriophages, Alexia Royer [et al.] 41

Molecular mechanisms of phage MDAΦ entry in Neisseria meningitidis, Clémence Mouville [et al.] 42

Characterization of new molecular partners involved in filamentous phage infection., Callypso Pellegri [et al.] 43

A novel strategy evolved by bacteriophage T5 to prevent E. coli general stress response, Nicolas Ginet [et al.] 44

Bacteriophage translocation across the intestinal barrier in inflammatory bowel disease, Clara Douadi [et al.] 45

Determining the main causes of phage resistance in clinical strains of Pseudomonas aeruginosa., Clarisse Plantady [et al.] 47

The blood virome in Crohn's disease, Quentin Lamy-Besnier [et al.] 48

Assessment of the carrier state life cycle in the lactic acid bacterium Oenococcus oeni following infection by distinct virulent phages, Amel Chaïb [et al.] 49

TBD, Stefanie Barbirz 50

RNA-based defense systems in the interactions of Clostridioides difficile with phages, Olga Soutourina 51

Innate immunity: the bacterial connection, François Rousset 52

Molecular mechanisms of phage MDAΦ entry in Neisseria meningitidis, Clémence Mouville [et al.] 53

Estimations of phage resistance and its impact on phage therapy against Pseudomonas aeruginosa respiratory infections, Ngoc Minh Chau Nguyen [et al.] . . . 54

Genetic screen to isolate bacterial mutants resistant to the toxicity of bacteriophage T5 genes, Godfred Morkeyile Annor [et al.] 55

Label free dynamic imaging: application to phage infected bacteria, Martine Boccara [et al.] . . . 56

DNA methylation levels and spontaneous mutations in E. coli lambda phage., Magali Ventroux [et al.] . . . 57

Determining the scale and rates of adaptation of methane-oxidising bacteria in response to virus interaction in soil, Huaiyu Wang [et al.] . . . 58

Tackling Multidrug-Resistant E. coli: A Dual Strike Approach using a phage cocktail and a host-targeting molecule, Devon Conti . . . 60

Isolation and characterisation of bacteriophages targeting Klebsiella pneumoniae, Vincent Fontaine [et al.] . . . 62

Development of virus-mediated nitrification inhibition, Felana Andrianjakarivony [et al.] . . . 63

Characterization of RNA viruses from plant roots and rhizosphere soil, Yuanyuan Zhang [et al.] . . . 64

Gram-negative Membrane Mimics and Other Tools to Study Host-Bacteriophage Interactions, Charles Arnaud [et al.] . . . 65

Application en Thérapie et Biotechnologie **67**

Optical trapping for the SUperfast Photonic detection of bacterioPhage Lysis (SUPPLY), Nicolas Villa [et al.] . . . 68

Antigen self-anchoring onto bacteriophage T5 capsid-like particles for vaccine design, Pascale Boulanger [et al.] . . . 70

Engineered phages as a potential solution to treat Clostridioides difficile infections?, Louis-Charles Fortier [et al.] . . . 71

Selection of therapeutic anti-Staphylococcus phages: in-depth in vitro characterization and in silico study of putative bacterial determinants of phage activity, Camille Kolenda [et al.] . . . 72

CRISPRi-seq identifies genes enhancing phage lysin Cpl-1 susceptibility in S. pneumoniae, Hugues De Villiers De La Noue [et al.] . . . 74

Current and future clinical phage product quality control in Belgium., Mathieu De Jode [et al.] . . . 75

Anti-P. aeruginosa phage development for clinical applications, Cindy Fevre [et al.] 76

Phage therapy and multidrug-resistant Escherichia coli: Isolation, selection, production of therapeutic phages and demonstration of their in vivo effectiveness in gut decolonization, Floriane Laumay [et al.] 77

Phages for Infections in Israel – The Israeli phage therapy center, Ran Nir-Paz . 79

Investigations of the role of neutrophils and macrophages in immunophage synergy during experimental pulmonary phage therapy, Sophia Zborowsky [et al.] 80

Isolation and Characterization of New Bacteriophages Against Staphylococcal Clinical Isolates from Diabetic Foot Ulcers, Lucile Plumet [et al.] 81

Caractérisation rapide de suspensions phagique par interférométrie. Partie 2 : vers la sécurisation en cours de process de purification., Benjamine Lapras [et al.] 82

Rapid characterization of phage suspensions by interferometry. Part 1: A comparison of particle quantification to the reference method (titration)., Léa Usseglio [et al.] 84

Characterization and genome analysis of novel bacteriophages vKpIN31 and vKpIN32, with lytic and anti-biofilm potential against Hospital acquired multidrug-resistant Klebsiella pneumoniae in Dakar, Senegal., Issa Ndiaye [et al.] 86

ENHANCING PHAGE THERAPY SAFETY: RELIABLE AND SENSITIVE PHAGE GENOME ANNOTATION WITH RTOOLS2 HIGH-THROUGHPUT PIPELINE, Antoine Culot [et al.] 88

Phagothérapie : analyse des risques et gestion sécurisée des bactériophages à l'hôpital, Manon Mennecier [et al.] 89

TOPICAL DERMAL PLATFORM FOR THE CO-DELIVERY OF CURCUMIN AND STAPHYLOCOCCUS AUREUS PHAGES, Carole Armal [et al.] 90

Evaluation of the activity of combinations of anti-Staphylococcus aureus phages, Camille Kolenda [et al.] 92

Phage-mediated biocontrol against plant pathogenic bacteria: a model of collaborations between academics and applied research, Nicolas Taveau [et al.] 94

Phage-mediated biocontrol in human health: a model of collaborations between academics and applied research, Marie Graindorge [et al.] 96

Developing the next generation of anti-staphylococci bacteriophage-based biomaterials, Emilie Cenraud [et al.] 97

Engineering Staphylococcus aureus phage K genome using CRISPR-Cas9, Kandas Traore [et al.] 98

Liste des participants **98**

Liste des auteurs **101**

Ecologie et Evolution

Genomics-based prediction of the phage-bacteria interactions at the species level

Hugo Vaysset * [1], Baptiste Gaborieau [2], Florian Tesson [1], Inès Charachon [3], Nicolas Dib [3], Juliette Bernier [3], Tanguy Dequidt [1], Héloïse Georjon [1], Olivier Clermont [3], Pascal Hersen [4], Laurent Debarbieux [1], Jean-Damien Ricard [5], Erick Denamur [3], Aude Bernheim† [1]

[1] Institut Pasteur – Institut Pasteur de Paris, Institut Curie, PSL Research University, CNRS, AgroParisTech, INRA - Université Paris-Saclay – 27, rue du Docteur Roux, 75015 Paris, France
[2] Assistance publique - Hôpitaux de Paris (AP-HP) – APHP-INSERM – NA, France
[3] Infection, Anti-microbiens, Modélisation, Evolution – Institut National de la Santé et de la Recherche Médicale, Université Paris Cité, Université Sorbonne Paris nord – Faculté de médecine Paris Diderot Paris 7 - site Bichat - 16 rue Henri Huchard 75890 Paris Cedex 18, France
[4] Institut Curie (UMR168) – Laboratoire Physico-Chimie Curie [Institut Curie] – 11 rue Pierre et Marie Curie75005 Paris, France
[5] Assistance publique - Hôpitaux de Paris (AP-HP) – AP-HP – NA, France

This ongoing arms race between prokaryotes and their viruses has led bacteria to develop diverse ways to resist phage infections. Phage bacteria interactions are thus complex and difficult to predict. For example, even if two bacterial strains are phylogenetically closely related, they can be susceptible to different phages. However major progress has been made in recent years to identify genomics drivers of phage bacteria interactions including the discovery of > 100 novel anti-phage systems. We thus set out to establish how much can be understood and predicted about phage bacteria interactions at the species level from DNA sequences. We systematically assessed the susceptibility of 403 natural, phylogenetically diverse, Escherichia strains to 96 bacteriophages from 19 distinct viral genera, resulting in an interaction matrix of 38,688 interactions. We characterized the genomics traits involved in these interactions and show that most phage-bacteria interactions can be explained by adsorption factors that are strongly linked to the strain a given phage was isolated on. We used genomics traits and the interaction matrix to train algorithms to predict phage-bacteria interactions that can pinpoint which interactions can be predicted well and where future research should focus on. Finally, we established a strategy to design tailored phage cocktails and assessed their effect on a collection of a 100 pathogenic E. coli. Altogether, this work brings new, quantitative, insights on the understanding of phage-bacteria interactions at the species level and pave the way for the use of predictive algorithms in phage therapy.

Mots-Clés: Spectre d'hôte, E. coli, Génomique

*Intervenant
†Auteur correspondant: aude.bernheim@pasteur.fr

The diversity and mobilization potential of P4-like phage satellites

Jorge Moura De Sousa * [1], Eduardo Rocha [1]

[1] Université de Paris, Institut Pasteur, CNRS UMR3525, Microbial Evolutionary Genomics – Institut Pasteur de Paris – Paris, 75015, France

The mobilization of bacteriophages is exploited by mobile genetic elements called phage satellites. Phage satellites are small elements that lack components of the viral particle for autonomous transfer. Instead, they encode diverse and sophisticated mechanisms to hijack the particles of helper phages. Albeit known for decades, phage satellites have only recently been recognized as characteristic mobile elements, that can transduce chromosomal DNA and encode virulence factors or anti-phage defense systems. Phage satellites have also been shown to be surprisingly abundant and ubiquitous across bacterial species. We characterised the four main known families of phage satellites and are currently investigating further one of the families – P4-like satellites, for which we find more than 3500 elements in Enterobacteria. P4 was originally described to exploit the phage P2, but experiments have shown that it can also be mobilized by a few other phages. It is not known whether this is also the case for all P4-like satellites, and whether promiscuity in helper phages has an impact on the efficiency of mobilization of the satellite. Our analysis of P4-like elements and P2-like prophages suggest that certain sub-families of satellites are more likely to mobilize with certain helper phages, based on genetic and phylogenetic signatures. Our results shed light in the (co-)evolution of the satellites-phage relationship, and besides improving our characterization of phage satellites, also provide a basis to predict their potential for mobilization across different bacterial hosts.

Mots-Clés: Phage satellites, Comparative genomics, Coevolution, Ecology, Horizontal gene transfer

*Intervenant

Origins and rate of mutations in bacteriophage Lambda

Julien Lopez *† [1], Marina Elez‡ [1], Marianne De Paepe§ [1], Mahendra Mariadassou [2], Valentin Loux [2,3], Rafael Sanjuan [4]

[1] MICrobiologie de l'ALImentation au Service de la Santé – AgroParisTech, Université Paris-Saclay, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – Domaine de Vilvert 78352 JOUY-EN-JOSAS CEDEX, France
[2] MaIAGE – Université Paris-Saclay, INRAE, MaIAGE – 78350, Jouy-en-Josas, France
[3] BioinfOmics, MIGALE bioinformatics facility – Université Paris-Saclay, INRAE, BioinfOmics, MIGALE bioinformatics facility – 78350, Jouy-en-Josas, France
[4] Instituto de Biología Integrativa de Sistemas (I2SysBio) – Parc Cientific de la Universitat de València C/ Catedrático Agustín Escardino 9 46980 Paterna, Valencia, Espagne

A single study (Dove et al. 1968) estimated that the mutation rate of lambda is between 100 and 1000-fold higher than that of its host, *Escherichia coli* (2.7x10-8 to 2.2x10-7 per base replicated for lambda vs. 2.0x10-10 for *E. coli*). Because *E. coli* DNA polymerase III replicates both genomes it is unclear why the mutation rate of lambda is this high.
To investigate this, we re-examined lambda mutation rate using a recent genomic approach for rare mutation detection, Duplex Sequencing (DS, Kennedy et al. 2014). This method uses UMI (Unique Molecular Identifier) barcoding to associate reads from the two complementary DNA strands and thus reduces the error rate by 10+4-fold. We found that the substitution rate of lambda is 1.2x10-8 substitutions per nucleotide per replication (s/n/r). In addition, in MMR deficient cells the lambda mutation rate is 2.2-fold higher (2.6x10-8 s/n/r) than in WT cells. In comparison, *E. coli* MMR- cells have a 138-fold higher mutation rate compared to WT cells. These results indicate that MMR is inefficient on lambda genome. The distribution of mutations was found to be random and the mutation rate is even across the genome, implying the absence of selection and mutational hotspots.
We are currently developing a new genetic reporter system for the determination of the mutation rate by fluctuation test, with the aim of confirming the results obtained by DupSeq, but also to further study the mechanism explaining the MMR inefficiency.

Mots-Clés: lambda, mutation rate, mismatch repair, duplex sequencing

*Intervenant
†Auteur correspondant: julien.lopez@inrae.fr
‡Auteur correspondant: marina.elez@inrae.fr
§Auteur correspondant: marianne.depaepe@inrae.fr

Study of a potential antiphage system of the "abortive infection" type regulated by a non-coding RNA in Clostridioides difficile

Marion Saunier *† [1,2], Olga Soutourina‡ [1], Louis-Charles Fortier§ [2]

[1] AP-HP. Université Paris Saclay – Institute for Integrative Biology of the Cell (I2BC), Universitey Paris-Saclay, UniversitéParis-Sud, CNRS, CEA, 91400 Orsay, France – I2BC, UMR 9198, Bât 23A, Gif-sur-Yvette, France
[2] Université de Sherbrooke – 2500 boul. de l'Université, Sherbrooke, Québec J1K 2R1, Canada

Abortive infection (Abi) is a bacterial defense mechanism against bacteriophages, which induces bacterial cell death (or dormancy) before the infecting phage can complete its replication cycle, thus protecting the bacterial population. Many genes were predicted as Abi systems, however, their mechanism of action is often unknown. An *abi*-like gene has been identified in *Clostridioides difficile,* a human pathogen classified as a main cause of antibiotic-associated diarrhea. Using RNA sequencing and Northern blotting, our group revealed a non-coding RNA (ncRNA) called RCd22 in the intergenic region upstream of the *abi* gene. This potential Abi system is located within the phi027 prophage, conserved in most epidemic ribotype 027 strains like the R20291 strain. Our goal is to study the function and regulation of this predicted Abi system. In *C. difficile*, overexpression of the *abi* gene results in a slower growth in both liquid and solid medium. This bacteriostatic effect has also been observed in *Escherichia coli.* RT-qPCR experiments on a deletion mutant lacking RCd22 showed increased expression of the downstream *abi* gene, suggesting a negative regulatory effect of RCd22 on gene expression. Moreover, transcriptional fusions with the alkaline phosphatase gene showed a decrease in reporter gene expression in the presence of RCd22. Interestingly, co-expression of RCd22 in *cis* but also in *trans* showed a restoration of normal growth in liquid and solid medium. A MS2-Affinity Purification technique coupled with mass spectrometry allowed us to identify *in vivo* the interaction between the Abi-like protein and the MS2-tagged RCd22, suggesting a mechanism of regulation similar to type III Toxin-Antitoxin systems where the ncRNA interacts with the toxin to inhibit its toxic effect. The function of the Abi-like protein as a defense mechanism against phages, and its role in stress response or prophage maintenance are currently under investigation.

Mots-Clés: antiphage system, abortive infection

*Intervenant
†Auteur correspondant: marion.saunier@i2bc.paris-saclay.fr
‡Auteur correspondant: Olga.soutourina@i2bc.paris-saclay.fr
§Auteur correspondant: Louis-Charles.Fortier@USherbrooke.ca

Oxidative stress influences the evolution of a synthetic community of gut bacteria and bacteriophage

Coline Meynard-Doumenc * [1], Loïc Brot [1], Auguste Wolfromm [1], Luisa De Sordi [1,2]

[1] Microbiote, intestin et inflammation [CRSA] – Centre de Recherche Saint-Antoine – Faculté de médecine Pierre et Marie Curie – Site Saint-Antoine – 6ème étage - 27, rue Chaligny – 75571 PARIS cedex 12, France
[2] Paris Center for Microbiome Medicine – Commissariat à l'énergie atomique et aux énergies alternatives, Institut National de la Santé et de la Recherche Médicale, CHU Pitié-Salpêtrière [AP-HP], Sorbonne Universite, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – Paris, France

Reactive oxygen species (ROS) play a central role in the pathogenesis of inflammatory bowel diseases (IBD). These diseases are characterized by an altered intestinal microbiota. We hypothesised that ROS would affect the diversity of bacteria and their bacteriophages (phages) and designed a study aimed at identifying the impact of ROS-induced oxidative stress on the interactions between them.
We set up a reductionist *in vitro* model using a defined microbial community of three *Escherichia coli* strains and a virulent phage during continuous culture in chemostats. We mimicked oxidative stress by adding hydrogen peroxide and compared the evolution of this community by studying population dynamics and the profiles of bacterial resistance and phage host-range over time, in the presence or absence of stress.

Phage and bacterial populations co-existed over 10 days in both conditions. However, hydrogen peroxide impacted phages-to-bacteria ratios and the evolution of the phage host range. Indeed, phage populations decreased in concentration and evolved to be more specialist under oxidative stress compared to the control condition. In bacteria, we observed an increase in resistance to infection in the presence of oxidative stress. Finally, time-shift experiments coupled with genomic analysis highlight potential signatures of phage-bacteria arms race.
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.

Mots-Clés: coevolution, gut microbiota, oxidative stress, resistance

*Intervenant

Phage susceptibility in phytopathogenic Pseudomonas syringae strains from different ecological niches

Chloé Feltin * [1], Garneau Julian [2], Cindy Morris [1], Annette Bérard [3], Clara Torres-Barceló [1]

[1] Pathologie végétale, INRAE – Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) – 67 allée des chênes, CS 60094 84143 Montfavet, France, France
[2] Department of Fundamental Microbiology, University of Lausanne – Lausanne, Switzerland, Suisse
[3] INRA UMR CSE-EMMAH – Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) – Domaine Saint Paul – Site Agroparc, 84914 Avignon Cedex 9, France, France

The bacterial species complex *Pseudomonas syringae* is diverse, ubiquitous and its life history is linked to water. These phytopathogenic bacteria can cause up to 60% losses in many crops. In the context of bacterial canker disease on apricot trees, several strains belonging to different phylogroups of this species complex are involved. Moreover, recent research suggest that they are phylogenetically closer to strains found in the environment than to strains found on plants. In this study, we explore the potential impact of phages on *P. syringae* communities on apricot trees.
As the most abundant entities on the planet, phages play a major role in their ecosystems. Their dynamics are being extensively investigated in marine environments, soil and human intestines, but few studies have been carried out in agricultural environments.
P. syringae -specific phages were sampled in apricot tree soils, isolated and analysed. Twenty-five new phages were characterized genetically and phenotypically. In the sampled soils, we reveal a ubiquitous presence of phages and an extraordinary diversity, with 14 genera and 21 new species discovered. We tested the differences in sensitivity of *P. syringae* strains to phages depending on the ecological niche where they were isolated, i.e. apricot or non-agricultural environment. We measured the capacity of 23 phages to inhibit the growth of 29 *P. syringae* strains in liquid culture. We observed 2 generalist phages and a majority of phage-resistant bacteria. The phage-bacteria interaction network analysis would indicate a nested structure with no modules distinguishing phages specialised in *P. syringae* strains isolated from apricot from those isolated from non-agricultural environments. Thus, these preliminary results suggest a continuum of interactions and no geographic isolation of *P. syringae* phages. This is consistent with the ubiquitous ecology of *P. syringae* with no influence from geographical location. Bacterial genetic traits related to phages are currently being studied.

Mots-Clés: Bacteriophages, Pseudomonas syringae, agriculture, ecology, host range

*Intervenant

Advances in understanding the ecology of bacteriophages in cheese

Thomas Paillet [1], Julien Lossouarn [2], Quentin Lamy-Besnier [2], Marie-Agnès Petit [2], Eric Dugat-Bony *† [1]

[1] UMR SAYFOOD – Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, Palaiseau, France – Palaiseau, France

[2] MICrobiologie de l'ALImentation au Service de la Santé – AgroParisTech, Université Paris-Saclay, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – Domaine de Vilvert 78352 JOUY-EN-JOSAS CEDEX, France

The production of smear-ripened cheese relies on the successive development of various microorganisms including LAB, yeasts and ripening bacteria. Bacteria play a major role in milk acidification, allowing coagulation, and in the ripening process, where they contribute to sensory properties such as colour, odour and taste. Recent studies indicate that a viral community, mainly composed of bacteriophages, coexists with cellular microorganisms in this ecosystem, but its ecological significance remains to be elucidated. Here, we used both a cultivation-based approach and viral metagenomics to get novel insights into the bacteriophage ecology of a French smear-ripened cheese.
First, five virulent phages infecting four non-starter culture bacterial species, namely *Glutamicibacter arilaitensis*, *Brevibacterium aurantiacum*, *Psychrobacter aquimaris* and *Leuconostoc falkenbergense*, were isolated from cheese and characterized through genome sequencing, transmission electron microscopy and host-range determination. PCR detection was also used for evaluating their distribution within potential reservoirs of the cheese factory. Second, we applied metagenomics tools to assess both the dynamics of phages and bacterial groups on the cheese surface during maturation, and their persistence in ready-to-eat cheeses throughout the production years. We observed a clear shift in the phage community composition during maturation, with a reduced proportion of viral taxa associated with *Lactococcus* phages, subsequently replaced by phages targeting ripening bacteria belonging to *Brevibacterium*, *Glutamicibacter*, *Pseudoalteromonas*, and *Vibrio* genera. This dynamics was strongly associated with bacterial successions observed on the cheese surface. Additionally, we determined that a large core-virome composed of the most dominant phages was consistently detected in matured cheese across production years, indicating long-term persistence of the main phages in the cheese production environment.
Together, these results provide new insights into the microbial ecology of cheese, highlighting the importance of phages during the ripening process. It also offers new avenues of research to better control this key step of cheese production.

Mots-Clés: bacteriophage ecology, fermented food, metavirome

*Intervenant

†Auteur correspondant: eric.dugat-bony@inrae.fr

Induction of prophages by bacteriocins in Escherichia coli fecal isolates

Caroline Henrot *† [1,2], Laurent Debarbieux [1], Marie-Agnès Petit [2]

[1] Bactériophage, bactérie, hôte – Université Paris Cité, Microbiologie Intégrative et Moléculaire – Département de microbiologie - 25-28 rue du Docteur Roux 75724 Paris cedex 15, France
[2] MICrobiologie de l'ALImentation au Service de la Santé – AgroParisTech, Université Paris-Saclay, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – Domaine de Vilvert 78352 JOUY-EN-JOSAS CEDEX, France

Prophage induction is latent in the intestinal microbiota, as witnessed by the abundance of temperate bacteriophages found in human virome data. However, little is known about the inducers that are involved in this persistent induction. Bacteriocin-encoding genes are regularly identified in the genomes of intestinal bacteria. Some of these toxic molecules that drive bacterial competition are also known to activate the SOS-response regulator RecA, which is involved in the canonical prophage-induction pathway. Furthermore, some studies have shown that colicins and microcins induce stx prophages of O157:H7 *E. coli* strains. However, to which extent the production of bacteriocins in the digestive tract induce prophages from lysogens remains unknown.
Using a large collection of *E. coli* fecal isolates, we investigated the potential of bacteriocins as prophage inducers. First, we found that supernatants of bacteriocin producing strains trigger prophages induction in two out of the three lambda lysogens used to detect such event. Second, we identified a plasmid encoding a microcin cluster as sufficient to confer a prophage-inducing ability to a laboratory strain. The next step is now to further characterize the action and spectrum of this microcin on various lysogens, as well as the prevalence of this cluster in a large collection of intestinal strains. Finally, we aim to decipher the induction of prophages by this microcin in a murine model.
Overall, these results suggest that intestinal bacteriocin-producing strains might be involved in prophage induction of natural lysogens. More broadly, this work adds to our understanding of how bacterial competition may take part in shaping the gut virome.

Mots-Clés: prophage, induction, Escherichia coli, gut, bacteriocins

*Intervenant
†Auteur correspondant: caroline.henrot@ens-lyon.fr

Identifying novel virus families infecting nitrifying archaea and bacteria in soil

Sungeun Lee *† [1], Graeme Nicol *

[1], Christina Hazard * ‡ [1]

[1] Ecole Centrale de Lyon – CNRS : UMR5005 – 36 avenue Guy de Collongue, Ecully 69134, France

While the complexity of prokaryote communities in soil is well understood, there remains a paucity of studies characterising the impact of viruses on hosts, biogeochemistry and rates of ecosystem processes. Microbially-mediated nitrification, central to the global nitrogen cycle and responsible for fertiliser losses in soil, is performed by taxonomically and functionally restricted groups of autotrophic bacteria and archaea and has led them to become established model groups for linking diversity, evolution, ecophysiology and function in soil. However, the diversity and impact of virus infection on these organisms are unknown. To identify active interactions between nitrifying prokaryotes and viruses, the transfer of assimilated carbon from autotrophic prokaryotes to viruses was examined using DNA stable isotope probing (DNA-SIP) coupled with metagenomic analysis. Combining with a novel hybrid analysis using GC mol% fractionation enabled identification of novel virus families infecting ammonia oxidising archaea (AOA). However, to characterise those also infecting ammonia- and nitrite oxidising bacteria, a second approach used filtration of virus-like particles from nitrifying soil microcosms after differential inhibition to alleviate competition and increase abundance of viruses infecting non-inhibited groups. This dramatically increased the recovery of high-quality virus genomes, with 225 from those infecting nitrifiers, including 69 complete or near-complete genomes ranging in size between 34 and 212 kb, all representing novel virus families. Soil AOA viruses did not contain auxiliary genes associated with ammonia oxidation but were enriched in those implicated in copper sequestration. Infection of nitrifiers is a dynamic process suggesting that understanding virus interactions during nitrification may inform approaches for augmenting activity and associated nitrogen losses.

Mots-Clés: Soil, nitrification, metagenomes, viromes, ammonia, oxidising bacteria, ammonia, oxidising archaea, nitrite, oxidising bacteria

*Intervenant

†Auteur correspondant: sungeun.lee@ec-lyon.fr

‡Auteur correspondant: christina.hazard@ec-lyon.fr

Inferring the life-history traits of new viral variants from epidemiological and evolutionary dynamics

Wakinyan Benhamou * [1], François Blanquart [2], Marc Choisy [3], Choquet Rémi [4], Sylvain Gandon [4]

[1] Centre d'Ecologie Fonctionnelle et Evolutive – Ecole Pratique des Hautes Etudes, Centre National de la Recherche Scientifique, Institut de Recherche pour le Développement, Université de Montpellier – Centre National de la Recherche Scientifique - 1919 route de Mende - 34293 Montpellier CEDEX 5, France

[2] Centre interdisciplinaire de recherche en biologie – Labex MemoLife, Collège de France, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique – 11 place Marcellin Berthelot 75005 Paris, France

[3] Centre for Tropical Medicine and Global Health [Oxford, UK] – Oxford University Clinical Research Unit (OUCRU) 764 Vo Van Kiet, W.1, Dist.5, Ho Chi Minh City Vietnam, Royaume-Uni

[4] Centre d'Ecologie Fonctionnelle et Evolutive – Université Paul-Valéry - Montpellier 3, Ecole Pratique des Hautes Etudes, Centre National de la Recherche Scientifique, Institut de Recherche pour le Développement, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Institut Agro Montpellier, Université de Montpellier – Centre National de la Recherche Scientifique - 1919 route de Mende - 34293 Montpellier CEDEX 5, France

Evolutionary epidemiology theory can help understand the time-varying selection acting on new pathogen variants. In particular, the strength of selection acting on more transmissible and/or more virulent variants is expected to change with the availability of susceptible hosts in the population. This theoretical framework is adequate to infer the life-history traits of new variants during epidemics in various situations. Here, we show how to combine the analysis of the evolutionary dynamics with the epidemiological dynamics to infer key epidemiological quantities of new variants. Here, we use data from an evolution experiment carried out with bacteriophage

Mots-Clés: Life, history traits, Evolutionary epidemiology, bacteriophage

*Intervenant

Bacteriophages in battle – how an anti-phage system distinguishes friend from foe

Jakob T Rostøl * [1]

[1] Centre for Bacterial Resistance Biology, Imperial College – London, Royaume-Uni

To defend against parasites such as bacteriophages (phages), a wide diversity of immune systems are expressed in bacteria. They are often carried by mobile genetic elements within the bacterial host, including on phages. In this manner, the element protects both itself and its host against other parasites. For this, defence genes must avoid targeting the cognate element they are carried on, while also providing broad protection against foreign invaders. In this talk, we will discuss that multiple *Staphylococcus aureus* prophages, including 80α, express Tha (Tail-activated, HEPN domain-containing Anti-phage system), a defence system that has non-specific RNase activity and is triggered by conserved structural tail proteins of incoming phages. Intriguingly, after induction, Tha has the potential to also block 80α reproduction. To avoid autoimmunity, this system is regulated by a small overlapping gene previously thought to be the phage excisionase. Through this antisense gene configuration, Tha protects the prophage and its bacterial host against phage predation, but is turned off during 80α prophage induction to avoid autoimmunity. We will propose that, as a part of their life cycles, prophages confer immunity, but have developed mechanisms to evade recognition by their cognate immune systems upon prophage activation.

Mots-Clés: Phage, antiphage system

*Intervenant

Following "Mystery training" *: How a bacteriophage adapts to diverse multidrug-resistant genotypes of E. coli ST131

Elsa Dardalhon Beurrier *† [1,2], Floriane Laumay [3,4], Mathieu Medina [3,4], Camille Kolenda [3,4], Laurent Dortet [5], Richard Bonnet [6], Frédéric Laurent [3,4], Rémy Froissart‡ [1]

[1] Virostyle – Perturbations, Evolution, Virulence – 911 Av. Agropolis, 34394 Montpellier, France
[2] Université de Montpellier – Université Montpellier I – 163 rue Auguste Broussonnet - 34090 Montpellier, France
[3] Pathogénie des Staphylocoques – Staphylococcal Pathogenesis – Centre International de Recherche en Infectiologie – Centre International de Recherche en Infectiologie (CIRI)Equipe Pathogénie des StaphylocoquesFaculté de médecine Laennec7, rue Guillaume Paradin69 008 Lyon, France
[4] Institut des Agents Infectieux [Lyon] – Hospices Civils de Lyon – Hôpital de la Croix-Rousse - 103 grande-rue de la Croix-Rousse - 69004 Lyon, France
[5] Centre National de Référence Associé de la Résistance aux Antibiotiques [Hôpital Bicêtre AP-HP] – AP-HP Hôpital Bicêtre (Le Kremlin-Bicêtre) – 78 rue du Général Leclerc 94275 Le Kremlin-Bicêtre, France
[6] Centre National de Référence de la Résistance aux Antibiotiques [CHU Clermont-Ferrand] – CHU Clermont-Ferrand – CHU Clermont-Ferrand - Site Gabriel-Montpied, 58 Rue Montalembert, 63000 Clermont-Ferrand, France

Mechanisms of bacterial resistance to bacteriophages are numerous (receptor modifications/suppression, presence of restriction-modification systems, CRISPR, etc.) and resistant genes are not necessarily homologous. The ease of bacteriophage adaptation to a bacterial population composed of multiple genotypes might thus depend on the level of bacterial diversity and the selective cost associated to each interaction.
We chose to study the adaptation of a bacteriophage (*Tequatrovirus)* evolving on 47 pathogenic *E. coli* clinical isolates (fully sequenced) belonging to the sequence type 131 (prevalent in nosocomial and community-acquired multidrug-resistant infections) and harboring a large genetic diversity. Of the 47 bacterial isolates, ten were susceptible and 36 were resistant or intermediate to the ancestral bacteriophage (*i.e.* full, none or partial bacterial growth inhibition in presence of the bacteriophage, respectively). After an experimental evolution *in vitro* using the Appelmans protocol with 14 serial passages, the evolved bacteriophage population of lineage 1 showed an extended host spectrum: 38 strains became susceptible and 9 harbored an intermediate growth inhibition. This experiment was repeated 3 times independently and we observed an increase both in virulence and host-range of the three independent bacteriophage lineages compared to the ancestral population. We plan to test the host-range extension of the evolved bacteriophage populations over a larger panel of bacterial isolates in order to confirm their "generalist" phenotype and to study the cost of generalism. As a temporary and partial conclusion, our results

*Intervenant
†Auteur correspondant: elsa.beurrier@ird.fr
‡Auteur correspondant: remy.froissart@cnrs.fr

clearly showed that we were able to generate in a short scale of time several bacteriophage populations with extended host-spectrum and increased virulence.

(*) "Mystery Train" is an enduring classic blues song interpreted by E. Presley, The Doors, B. Dylan, etc.

Mots-Clés: Adaptation, Escherichia coli, Bacteriophage Evolution, Phage Therapy, Experimental Evolution, Host Range

Characterization of virus-host dynamics in anaerobic digesters under abiotic stress

Marion Coves* [1], Cédric Midoux [1,2], Mahendra Mariadassou [2], Olivier Chapleur [1], Laurent Mazéas [1], Ludwig Jardillier [3], Ariane Bize †‡ [1]

[1] Université Paris-Saclay, INRAE, PROSE – INRAE, Université Paris-Saclay – 92761, Antony, France
[2] Université Paris-Saclay, INRAE MaIAGE – Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – Bât. 233, Domaine de Vilvert - 78352 Jouy-en-Josas Cedex, France
[3] Université Paris-Saclay, IDEEV, ESE – AgroParisTech, Université Paris-Saclay, Centre National de la Recherche Scientifique – bat. 362 91405 ORSAY CEDEX, France

Viruses of microbes remain little explored in anaerobic digestion (AD) plants, where biowaste is valorised into methane-rich biogas. The AD process ecosystems are very sensitive to disturbance, leading to inhibition 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 studied 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 as a provirus activator. We monitored meaningful physico-chemical parameters over time. We also performed 16S rDNA metabarcoding targeting archaea and bacteria, and shotgun metagenomic sequencing of 30 selected metaviromes.

We first confirmed a significant impact of the tested stresses on biogas production. A total of 20 procaryotic genera showed significantly different dynamics compared to the control and their analysis revealed the inhibition of ecological successions in the presence of inhibitors.
Metavirome coassembly yielded 45,914 contigs predicted as putatively viral by VIBRANT, including 2,815 complete viral genomes. 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. After further filtering with two additional tools, VirSorter2 and CheckV, 230 contigs were further selected as highly reliable. Upon those, 132 in total showed significantly different dynamics compared to the control. To assess the activation of proviruses by the inhibitors, we considered integrase genes as a proxy for temperate viruses and calculated the ratios of temperate over virulent viruses, based on normalised read counts. No significant trend towards provirus activation was observed, suggesting that viruses, in our study, did not aggravate AD dysfunctions.

*Auteur correspondant: marion.coves@inrae.fr
†Intervenant
‡Auteur correspondant: ariane.bize@inrae.fr

Mots-Clés: viral ecology, anaerobic digestion, inhibition, disruption, provirus, provirus activation, abiotic factor

Diversité virale des eaux de baignade de la Seine

Alisa Langlais * [1], Céline Roose-Amsaleg [1], Achim Quaiser [1]

[1] Ecosystèmes, biodiversité, évolution [Rennes] – Université de Rennes, Institut Ecologie et Environnement, Centre National de la Recherche Scientifique, Observatoire des Sciences de l'Univers de Rennes – Bâtiment 14 - Université de Rennes 1 - Campus de Beaulieu - CS 74205 - 35042 Rennes Cedex - France, France

Actuellement, la qualité des eaux de baignade impose uniquement la mesure de 2 indicateurs bactériens de contamination fécale humaine. Or, les virus représentent l'entité biologique majoritaire sur Terre, étant estimés à 10^2 dans les eaux et ils sont surtout connus pour leur pathogénicité. Cependant, la majorité de ces virus aquatiques sont des bactériophages dont moins de 1% sont connus à ce jour. Appréhender leur diversité et leurs dynamiques représente un enjeu pour comprendre leurs implications au sein d'un écosystème comme la Seine.
L'ADN viral d'eaux brutes a été extrait de plusieurs façons puis séquencé par la technologie Illumina MiSeq. Les données brutes de séquençage ont été nettoyées ; les contigs assemblés par SPAdes et MetaviralSPAdes pour comparaison. Une famille des Microviridae, a été caractérisée en se basant sur son marqueur phylogénétique : la séquence codante pour la protéine de capside VP1.
La profondeur, la qualité et la contamination du virome étaient satisfaisantes. Au total, plus de 90% des taxons retrouvés dans les eaux brutes de la Seine représentaient des bactériophages. Parmi eux, les 2 principaux groupes étaient la classe des Caudoviricetes et la famille des Microviridae. Ces derniers dont les Gokushovirinae sont des bactériophages ubiquitaires de l'environnement. Les séquences de 11 nouveaux Gokushovirinae ont été annotées et pourraient représenter un indicateur de la dynamique virale des eaux de rivière.

Mots-Clés: Virome, Microviridae, Seine, Bioinformatique

*Intervenant

Tracking viral infection temporal dynamics after microbial resuscitation during seasonally dry soil rewetting using viromics and stable isotope metagenomics

Ella Sieradzki * [1], Alexa Nicolas [2], Jennifer Pett-Ridge [2,3,4], Jillian Banfield [2,5], Michiko Taga [2], Mary Firestone [2], Steven Blazewicz [3]

[1] EMG$_{L}yon - -EcoleCentraledeLyon - -36aveGuydeCollongue, Lyon69130, France$
[2] UCBerkeley – 2151 Berkeley Way, Berkeley, CA, États-Unis
[3] LLNL – Lawrence Livermore National Laboratory, CA, États-Unis
[4] UCMerced – 5200 Lake Rd, Merced, CA 95343, États-Unis
[5] LBNL – Lawrence Berkeley National Laboratory, CA, États-Unis

Viruses are highly active during ecosystem perturbations in aquatic systems, but these dynamics in soil ecosystems are unknown. Previous studies have shown that soil rewetting propels a succession of the microbial community with specific lineages growing and dying at different times. This taxonomic signature likely indicates involvement of viruses in mortality.
Here, we investigated lineage-specific virus-host dynamics in grassland soil following soil rewetting, when resident microbes are both resuscitated and lysed after a prolonged dry period.

To identify actively infecting viruses, we used a replicated time-series including a combination of viromes and stable isotope probing (SIP). We also characterized host succession via SIP-metagenomics using 18O-labeled water, and tracked the incorporation of the isotope into viruses.

Dry soil held a diverse but low biomass reservoir of virions, of which only a subset thrived following wet-up. We used isotope incorporation into viral and microbial DNA to characterize virus-host temporal dynamics. Viral richness decreased by 50% within 24 h post wet-up, while viral biomass increased four-fold within one week. Counter to recent hypotheses suggesting temperate viruses predominate in soil, our evidence indicates that wet-up is dominated by viruses in lytic cycles. We estimate that viruses drive a measurable and continuous rate of cell lysis, with up to 46% of microbial death driven by viral lysis one week following wet-up, resembling rates in marine systems that yield 20% of the dissolved organic carbon pool.
Thus, viruses contribute to turnover of soil microbial biomass and the widely reported CO2 efflux following wet-up of seasonally dry soils, with a potentially significant viral shunt that likely contributes to soil carbon sequestration.

Mots-Clés: Wetup, virome, SIP, mortality, succession, soil

*Intervenant

" This Training Is Bound for Glory"* : How a bacteriophage adapts face to diverse bacterial genotypes

Amandine Maurin * [1,2]

[1] Virostyle – Perturbations, Evolution, Virulence – IRD 911 avenue Agropolis 34394 Montpellier, France
[2] CNRS – UMR 224 MIVEGEC (IRD, UM, CNRS), Montpellier – 1919 route de Mende 34000 Montpellier, France

Mechanisms of adaptation of bacteriophages towards multiple strains of bacteria are various and depend not only on their ability to recognize receptors on the bacterial surface but also on multiple other intracellular interactions (restriction enzymes, DNA modification, CRISPR, etc). Due to those multiple selection pressures acting on bacteriophages, it is difficult to predict which function are targeted to allow adaptation.
To study bacteriophage adaptation to a polymorphic bacterial population, we experimented in vitro seven serial passages of one isolated and purified Tequintavirus bacteriophage (five independent lineages) against eight bacterial isolates *of* Salmonella enterica serotype Tenessee belonging to two sequence types (four isolates of ST319 and four of ST5018). While the ancestral bacteriophage was able to infect isolates of one sequence type, evolved populations expanded their host range (8/8 infected bacterial genotypes). Moreover, bacterial growth inhibition of evolved bacteriophage populations was maintained without appearance of resistant bacteria for more than 20 hours despite a 3-4 log dilution of the bacteriophage populations. After sequencing DNA from both ancestral and evolved populations, we observed parallel mutations in evolved populations toward modification of several genes such as the long tail fiber protein gene (potentially involved in interaction with the bacterial receptor), exo- and endo-nuclease (potentially involved in modification of mutation rate) as well as hydrolase (potentially involved in increase of virulence). Interestingly, adaptation seems to take place after point mutation accumulations but also after recombination with prophage genes.
For the sake of successful bacteriophage therapy, our results show the importance of in vitro evolutionary training taking into account the diversity of bacteria isolated in situ prior to the application of therapeutic bacteriophages.

Mots-Clés: Phages Evolution, Salmonella, Adaptation, Host, range, Virulence, Sequencing, Recombination, Mutations, Tail fibers, Reverse genetic

*Intervenant

Spotlight on the viral diversity of fermented vegetables using viral metagenomics

Julia Gendre *† [1], Claire Le Marrec [2], Stéphane Chaillou [3], Sophie Landaud [1], Éric Dugat-Bony [1]

[1] UMR SAYFOOD – AgroParisTech, INRAe - Université Paris-Saclay – 22 Place de l'agronomie 91120 Palaiseau, France
[2] UMR OENOLOGIE – Institut des Sciences de la Vigne et du Vin (ISVV), Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) – Institut des Sciences de la Vigne et du Vin, Villenave d'Ornon, France
[3] MICALIS INSTITUTE – Université Paris-Saclay, INRAE, AgroParisTech – Domaine de, All. de Vilvert, 78350 Jouy-en-Josas, France

Fermented vegetables are traditionally made using natural microorganisms found in the raw ingredients. The bacterial and fungal communities of fermented vegetables are well known, which is not the case for phage communities. Previous studies have identified various phages infecting different bacteria in sauerkraut, revealing the complexity of phages in this ecosystem, and metagenomic analysis highlighted the predominance of uncharacterized phages. Our project aims to provide a comprehensive description of the viral communities in fermented plant-based foods (sauerkraut, fermented carrots, and fermented turnips) to better understand their role in these microbial ecosystems.
The viral fraction was purified, quantified by epifluorescence microscopy and determined through a viral metagenomics approach involving nucleic acid extraction, DNA and RNA amplification and high throughput sequencing. A bioinformatic pipeline was established to process raw reads into viral operational taxonomic units (vOTUs). Representative sequences from each vOTU were analyzed to predict viral contigs and host.

Based on epifluorescence microscopy, we estimated the concentration of viruses at 5 x 10^7 particles/g of sample. Viral metagenomics analysis identified viral contigs with sequence homology to known phages, including those infecting *Lactobacillus brevis*, a common species in fermented vegetables. Some contigs were predicted to be viral, albeit without strong homology to known viruses, but with a bacterial host prediction, mainly belonging to lactic acid bacteria typically found in vegetable fermentation.
In conclusion, this study offers insights into the composition of viral communities in different fermented vegetables, focusing on extracellular viral particles. The findings suggest that several phages target the dominant lactic acid bacteria involved in the fermentation process, potentially influencing bacterial successions in the ecosystem. Understanding the ecological roles of these phages is crucial for controlling their activity and ensuring the quality of the fermentation process.

*Intervenant
†Auteur correspondant: julia.gendre@inrae.fr

Mots-Clés: Fermented vegetables, bioinformatic, epifluorescence microscopy, metagenomic, phages ecology

Phage-plasmids produce novel types of plasmids and phages and drive gene flow between them

Pfeifer Eugen *† [1], Eduardo Rocha [1]

[1] Institut Pasteur [Paris] – Institut Pasteur, Université de Paris Cité, CNRS UMR3525, Microbial Evolutionary Genomics, Paris, France – 25-28, rue du docteur Roux, 75724 Paris cedex 15, France

Phages and plasmids are distinct classes of mobile genetic elements (MGEs), each employing different mechanisms for DNA transfer: plasmids use conjugative pili, while phages use infective virions. Yet, there are also phage-plasmids (P-Ps), a special group of temperate phages that transfer through viral infection but replicate as plasmids in the host (without integrating the chromosome). This blurs the definitions of phages and plasmids.
Recently, we demonstrated the prevalence of P-Ps, and some of them even carry antibiotic resistance genes, spreading them as phages by generating resistant lysogens. The presence of nearly identical resistance genes on plasmids suggests recombination events, sparking our interest in studying their relatedness and genetic exchanges.
In our current work, we analyzed 3585 phages, 20274 plasmids and 1416 P-Ps and assessed their gene repertoire relatedness using protein-sharing networks. Our results reveal that P-Ps are centered at the genetic interface of phages and plasmids. For certain P-Ps, we identified highly related plasmids and (integrative) prophages, which we used as a case study to deeply analyze the phylogenety. Some of these P-Ps lost essential phage genes (e.g. encoding tails or lysins) and acquired genetic regions facilitating mobilization (oriTs or relaxases), ultimately evolving into plasmids capable of moving via conjugation. Furthermore, although rarely observed, some P-Ps were found integrated within host chromosomes, conclusively giving rise to novel prophages. To assess gene flow between different MGEs (phages, plasmids and P-Ps), we searched for recombining genes by searching for very similar genes in highly divergent genomes. We found P-Ps to promote exchanges between phages and plasmids, which typically rarely recombine, including transfers of antibiotic resistances and defense genes.
In conclusion, P-Ps play a key role in exchanging traits across very different MGEs and their hosts, while facilitating the transition of one type of element into another.

Mots-Clés: Phage, plasmids, phages, plasmids, gene flow, recombination, evolution of MGEs

*Intervenant

†Auteur correspondant: eugen.pfeifer@pasteur.fr

The virulent Krappator 27 phage selects for unique genomic changes in the lactic acid bacterium Oenococcus oeni.

Florencia Oviedo Hernandez * [1], Amel Chaïb [1], Olivier Claisse [1], Claire Le Hénaff - Le Marrec * † [1]

[1] Unité de Recherche Œnologie [Villenave d'Ornon] – Université de Bordeaux, Institut des Sciences de la Vigne et du Vin (ISVV), Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – 210 Chemin de Leysotte - CS50008 - 33882 Villenave d'Ornon, France

The lactic acid bacteria *Oenococcus oeni* is an essential player in the winemaking process. Previously, oenophages have been isolated and characterized suggesting that phages are an unexplored actor in the wine environment. In this study, we investigated how the bacterial genome evolves during phage infection. Co-evolution assays were carried out in MRS Broth in four replicates. The experimental design included a total of 19 transfers (around 95 generations) for each evolved and coevolved culture. Bacterial and phage populations were enumerated at the early steps of the experiments (transfers 1 to 5) and at the end (transfer 16 to 19). The phage population decreased significantly at the final transfers in 3 of 4 replicates and extinction was observed in one culture. Individual colonies were isolated at relevant steps (T3, T16 and T19) and their resistance towards Krappator 27 was assessed. The spectra were extended to the virulent phage Vinitor162 and the ex-temperate phage OE33PA1,2,3, which are expected to use different receptors in *O. oeni*. Clones with distinct panel of resistance/sensitivity were selected over time. At early transfer a diversity of resistant phenotypes were found, while at the later transfers only one type of resistance was found, where clones were resistant to the original phage Krappator 27 and OE33PA but completely sensitive to Vinitor 162, suggesting that selection of more fitted clones took an import role at the end of the experience. Representative colonies were further selected and their genomes sequenced. Through BreSeq analyses, we observed an array of mutations in cell wall macromolecule- encoding genes, and their analyses will give clues to identify the receptor on the surface of the host cell. 1, Chaïb et al., 2022 Int J Food Microbiol. 2022;383:109936 ; 2, Chaïb et al., 2019, Appl Environ Microbiol. 85(19):e00997-19; 3, Philippe et al., 2021, Front Microbiol. 2021;11:596541.

Mots-Clés: Coevolution, oenophages, lactic acid bacteria, evolution, cell wall receptors

*Intervenant

†Auteur correspondant: clehenaff@enscbp.fr

Structure et Assemblage

Cryo-electron tomography study of bacterial cell wall perforation by bacteriophage T5

D'acapito Alessio *† [1], Claudine Darnault [1], Lorenzo Gaifas [1], Guy Schoehn [1], Emmanuelle Neumann‡ [1], Cécile Breyton§ [1]

[1] Institut de biologie structurale – Centre National de la Recherche Scientifique, Institut de Recherche Interdisciplinaire de Grenoble, Université Grenoble Alpes – Institut de Biologie Structurale, 71 avenue des martyrs, CS 10090, 38044 Grenoble Cedex 9, France

Bacteriophages are fascinating nanomachines that employ highly specific and remarkably efficient molecular machineries to infect their bacterial hosts.
While the mechanisms behind the contraction of the tails of myophages, as phage T4, have been well characterized, very little is known about the molecular mechanisms of the infection of siphophages, as phage T5. We have already elucidated the 3D structure at atomic resolution of the distal tail complex of the T5 phage, before and after interaction with its *E. coli* receptor FhuA reconstructed into a nanodisc, using single particle cryo-electron microscopy. From this work, we proposed a mechanism that explains how host recognition triggers the first steps of infection, the opening of the phage tail, its anchoring to the outer membrane and the insertion of a channel into the outer membrane.

However, the gram-negative *E. coli* cell wall is far from being a simple nanodisc. The mechanism of inner membrane perforation and the identity of the proteins (phage or host borne) involved in the channel going through the entire cell wall and regulating DNA ejection remain a mystery.
My PhD project aims, using cryo-electron tomography (cryo-ET), to investigate the structure of the T5 phage channel perforating the bacterial cell wall close from *in vivo conditions*, using *E. coli* minicells. The use of minicells allows to image the infection in the electron microscope: indeed, entire *E. coli* cells are too thick for imaging without additional sophisticated sample preparation. Image processing is now ongoing on a small preliminary data set of tomograms to determine the architecture of the perforation apparatus of phage T5.

Mots-Clés: Cryo, Electron Microscopy, Cryo, Electron Tomography, T5 bacteriophage, Infection, Sructure

*Intervenant
†Auteur correspondant: alessio.dacapito@ibs.fr
‡Auteur correspondant: Emmanuelle.NEUMANN@ibs.fr
§Auteur correspondant: cecile.breyton@ibs.fr

Structural insights into host-binding machineries of mycobacteriophages revealed by AlphaFold2

Christian Cambillau [1,2,3], Adeline Goulet *† [1]

[1] Laboratoire d'ingénierie des systèmes macromoléculaires – Aix Marseille Université, Centre National de la Recherche Scientifique – 31 Chemin Joseph Aiguier 13402 MARSEILLE CEDEX 20, France
[2] University College Cork – School of Microbiology, Irlande
[3] AlphaGrafix – Entreprise privée – Formiguères, France

The large public collection of nearly 2,300 sequenced mycobacteriophages (phages of mycobacteria) offers great opportunities to better understand the diversity of phages and their interactions with their hosts. Yet, the architecture of their host-binding machineries and the manner with which they bind to the mycobacterial envelope at the onset of the viral infection remain remarkably elusive. Therefore, we explored the structure of host-binding machineries from mycobacteriophages infecting different *Mycobacterium* hosts using AlphaFold2. Our results provided unprecedented structural insights on these modular and structurally diverse assemblies that contain, in addition to a *bona fide* receptor-binding protein (RBP), one or more additional proteins likely also involved in host binding. Moreover, we found that these RBPs and potential additional host-binding proteins are composed of canonical carbohydrate-binding modules and lectin-like domains, which likely bind to polysaccharides, as well as uncommon polyglycine-rich domains (PGD). Interestingly, PGDs had only been observed in five proteins including the tail fiber adhesin of the *Salmonella* phage S16 in which one PGD is involved in host binding. Since these PGDs in mycobacteriophages expose hydrophobic residue patches, we hypothesize that they may interact with lipids of the mycomembrane. Our work paves the way for further structural and functional characterization of these machineries, which is important to better understand phage biology as well as develop tools for mycobacterial infection detection and treatment.

Mots-Clés: host, binding machineries, AlphaFold2, mycobacteria

*Intervenant
†Auteur correspondant: adeline.goulet@univ-amu.fr

Assembly regulation of pb6, siphophage T5 Major Tail Protein

Charles-Adrien Arnaud [1], Claudine Darnault [2], Undina Guillerm [2], Yong Wang [3], Corinne Vivès [2], Kresten Lindorff-Larsen [3], Paul Schanda [2], Cécile Breyton [*† 1]

[1] IBS – CNRS, UGA, CEA – Grenoble, France
[2] IBS – CNRS, UGA, CEA – Grenoble, France
[3] Univ Copenhagen – Copenhagen, Danemark

Bacteriophages are remarkable nanomachines that evolved to specifically target their host, efficiently highjack the bacterial machinery to produce and release, at the end of the infection cycle, dozens to hundreds of new virions. In the case of long-tailed phages, which represent a large majority of known phages, assembly pathways independently produce DNA-filled capsids and functional tails that ultimately interact to form a viable virion in the host cytoplasm. These assembly pathways are highly and tightly regulated. In particular, the Major Tail Protein polymerises only around the Tape Measure Protein, once the assembly nucleus has formed, to form the tail tube. We have previously determined the structure of siphophage T5 Major Tail Protein pb6 (1). When overexpressed, the small proportion of soluble protein produced is extremely stable and can be concentrated up to 100 mg/mL while remaining monomeric (1). This suggests a mechanism that prevents the protein to self-oligomerise without its tail partners. Here, we show that the pb6 monomer in solution is in equilibrium between a very minor (5%) folded state and a partially unfolded state (95%), as determined by NMR and retrieved by molecular dynamics simulations. When the folded state is artificially stabilised by a disulfide bond, the mutated protein is much less stable and readily forms tube. Moreover, for the wild type protein, self-oligomerisation is triggered by the addition of preformed tubes, as confirmed by negative stain electron microscopy. Taken altogether, our data show that the partial unfolding of the wild type protein in solution prevents self-polymerisation in physiological conditions, allowing tube formation only in the presence of a folded partner, as is the case in the tail assembly pathway or by the addition of preformed tubes. (1) Arnaud et al, Nat Commun. 2017 Dec 5;8(1):1953. doi: 10.1038/s41467-017-02049-3.

Mots-Clés: Phage T5, Major Tail Protein, Assembly regulation

[*]Intervenant
[†]Auteur correspondant: Cecile.Breyton@ibs.fr

Mechanisms of assembly and evolution of the dsDNA gatekeeper from the tailed viruses-herpesviruses lineage

Igor Orlov [1], Stéphane Roche [2], Sandrine Brasiles [2], Natalya Lukoyanova [3], Sophie Zinn-Justin [4], Marie-Christine Vaney [5], Paulo Tavares * [2], Elena Orlova [3]

[1] Centre for Integrative Biology - CBI – Institut de Génétique et de Biologie Moléculaire et Cellulaire – Université de Strasbourg, Illkirch, France, France
[2] I2BC – Université Paris-Saclay, CEA, CNRS – Bâtiment 14b 1 avenue de la Terrasse 91198 Gif-sur-Yvette, France
[3] Birkbeck College [University of London] – Malet St, London WC1E 7HX, Royaume-Uni, Royaume-Uni
[4] I2BC – Université Paris-Saclay, CEA, CNRS – CEA, Saclay, France
[5] Institut Pasteur – Institut Pasteur – 25 avenue du Dr Roux, Paris, France

Numerous viruses assemble a precursor procapsid structure for subsequent encapsidation of the viral genome. Members of the tailed viruses-herpesviruses lineage package their double-stranded DNA through a specialized portal vertex into the procapsid. Genome packaging termination is followed by reversible closure of the portal system. Then a tail is attached to the portal vertex of prokaryotic viruses or a tegument surrounds the capsid of herpesviruses. This adaptation for infection of bacteria/archaea or of eukaryotic cells, respectively, marks the divergence step within the lineage.
We will present studies on the structure and function of the portal dsDNA gatekeeper of bacteriophage SPP1. Comparison of the gatekeeper structure at the post-DNA packaging state (1) with assembly-naïve structures of its individual components (2) provides insight on the conformational changes ensuring sequential interaction of the proteins building the gatekeeper (1). The resulting assembly model allows rationalizing extensive mutagenesis and functional data (1-4). Present knowledge on the SPP1 gatekeeper combined with comparative structural biology of its proteins reveals branching points of herpesviruses, short tailed-, and long tailed viruses evolution.

(1) Orlov et al (2022) *Nat Commun* 13, 7283.

(2) Lhuillier, et al (2009) *Proc. Natl. Acad. Sci. USA* 106, 8507-8512

(3) Chaban et al (2015) *Proc. Natl. Acad. Sci. USA* 112, 7009-7014.
(4) Isidro et al (2004) *Virology* 322, 253-263.

Mots-Clés: viral assembly, genome packaging

*Intervenant

Probing the world of phage specificity: a glimpse into receptor binding protein structures and their development as diagnostic bio-probes

Matthew Dunne *† [1]

[1] ETH Zurich – Rämistrasse 101, 8092 Zürich, Suisse

Bacteriophages, nature's bacterial predators, are celebrated for their extraordinary precision in binding to bacterial targets. This precision is achieved through specialized receptor binding protein (RBP) complexes located on the phage particle. These RBPs can be categorized into two distinct groups: tail fibers (TFs) and tailspike proteins (TSPs), each distinguished by its unique morphology. TFs are elongated and slender fibrous proteins, while TSPs are short, stocky, and often equipped with enzymatic capabilities targeting specific surface structures. As the initial point of contact with a bacterial host, RBPs serve as the critical gatekeepers for all phage infections, with their binding range fundamentally determining the phage's host range.
This presentation of work performed at ETH Zurich delves into the intricate world of phage RBP structures, placing special emphasis on engineering RBPs as diagnostic bio-probes for various clinical and foodborne pathogens. It also offers a glimpse into the design principles of RBP engineering and how this may contribute to the advancement of phage-based therapeutics.

As a prime illustration of our research, we've uncovered the intriguing structure of the *Salmonella* phage S16 long tail fiber (LTF), consisting of two distinct proteins: a long rod-like gp37 homotrimer and a "polyglycine sandwich" adhesin (gp38), which caps the gp37 fiber tip, playing a pivotal role in host selectivity. We've harnessed the remarkable binding range of the S16 LTF to develop an innovative enzyme-linked LTF assay (ELLTA). This assay combines LTF-coated magnetic beads with horseradish peroxidase-conjugated LTF, facilitating quick and sensitive detection of this critical foodborne pathogen. Additionally, by conjugating tail fibers onto latex beads, we developed an agglutination-based assay for detecting *Burkholderia pseudomallei*, the causative agent of melioidosis in endemic regions of Thailand and Southeast Asia.

Mots-Clés: Phage, RBP, bioprobe

*Intervenant
†Auteur correspondant: matthew.dunne@hest.ethz.ch

Interaction Phage-hôte

Characterization of a (p)ppApp synthetase belonging to a new family of polymorphic toxin associated with temperate phages

Julie Viala * [1], Julia Bartoli [1]

[1] Laboratoire d'ingénierie des systèmes macromoléculaires – Aix Marseille Université, Centre National de la Recherche Scientifique – Batiment IBSM 31 Chemin Joseph Aiguier 13402 MARSEILLE CEDEX 20, France

Polymorphic toxins (PTs) are a broad family of toxins involved in interbacterial competition and pathogenesis. PTs are modular proteins that are comprised of a conserved N-terminal domain responsible for its transport, and a variable C-terminal domain bearing toxic activity. Although the mode of transport has yet to be elucidated, a new family of putative PTs containing an N-terminal MuF domain, resembling the Mu coliphage F protein, was identified in prophage genetic elements. The C-terminal toxin domains of these MuF PTs are predicted to bear nuclease, metallopeptidase, ADP-ribosyl transferase and RelA_SpoT activities. In this study, we characterized the MuF-RelA_SpoT toxin associated with the temperate phage of *Streptococcus pneumoniae* SPNA45. We show that the RelA_SpoT domain has (p)ppApp synthetase activity, which is bactericidal under our experimental conditions. We further determine that the two genes located downstream encode two immunity proteins, one binding to and inactivating the toxin and the other detoxifying the cell via a pppApp hydrolase activity. Finally, based on protein sequence alignments, we propose a signature for (p)ppApp synthetases that distinguishes them from (p)ppGpp synthetases.

Mots-Clés: toxin, antitoxin, immunity protein, (p)ppGpp, (p)ppApp, modified nucleotide, MuF, temperate phage

*Intervenant

Bacteriophage 933W Can Reach Brain Tissues of Escherichia coli Monocolonized Mice, Provided its stx Eukaryotic Promoter is Functional

Marie-Agnès Petit * [1], Alipio Pinto [2], Sylvie Rabot [1], Carla Rivero [3], Paulo Quiroz [4], Maité Muniesa [4], Marina Palermo [5], Jorge Goldstein [5], Leticia Bentancor [3]

[1] MICrobiologie de l'ALImentation au Service de la Santé – AgroParisTech, Université Paris-Saclay, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – Domaine de Vilvert 78352 JOUY-EN-JOSAS CEDEX, France

[2] Universidad de Buenos Aires [Buenos Aires] – Viamonte 430/44 C1053ABJ, Ciudad de Buenos Aires, Argentine

[3] Instituto de Estudios para el Desarrollo Productivo y la Innovación, Universidad Nacional de José Clemente Paz. – Buenos Aires, Argentine

[4] University of Barcelona – Gran Via de les Corts Catalanes, 585, 08007 Barcelona, Espagne

[5] Consejo Nacional de Investigaciones Científicas y Técnicas [Buenos Aires] – Avda. Rivadavia 1917 - CP C1033AAJ - Cdad. de Buenos Aires, Argentine

Hemolytic Uremic Syndrome (HUS) is a consequence of Shiga toxin-producing Escherichia coli infections. This toxin is encoded on various bacteriophages, the most studied being 933W. A non-pathogenic strain of E. coli (C600) lysogen for the bacteriophage 933W (C600Φ933W), is able to induce intestinal, renal and brain damage associated with HUS development, showing that no other gene outside the prophage is needed for pathogenicity (del Cogliano et al., Front. Microbiol. 2018). Moreover, a pr1 eukaryotic promoter was identifed upstream of the stxA2 gene on 933W. Using plasmid constructions, a TATA box mutation within this putative eukaryotic stx2 promoter (933W:Δpr1) was found to prevent expression of Shiga toxin in eukaryotic cells (Bentancor et al., PLoS One, 2013). In the present work, we searched for the role of this pr1 eukaryotic promoter. For that, the TATA box mutation was inserted into the prophage genome of strain C600Φ933W,while maintaining the nearby bacterial promoter of stxA2. Germ free mice were colonized either with C600Φ933W or an isogenic strain carrying the mutation (C600Φ933W:Δpr1). In both groups of mice, 933W phage DNA was detected in sera samples by PCR, indicating a certain level of passage of particles in the blood. Analysis of intestinal, liver and kidney histology of these mice did not reveal differences between C600Φ933W vs. C600Φ933W:Δpr1, suggesting that damage to these tissues is not dependent on the pr1 promoter. However, we observed a significant difference in the detection of phage particles and Stx toxin in brains of mice infected with C600Φ933W vs. C600Φ933W:Δpr1. In addition, immunofluorescent markers of brain damage (GFAP and MBP) also differed in mice inoculated with the two strains. These results support the hypothesis that Stx expression by pr1 prmoter in the host cells could be important in HUS development.

*Intervenant

Mots-Clés: Prophage STEC pathogen

The phages which don't kill Xanthomonas hortorum pv. vitians, doesn't make it stronger: molecular determinants and trade-off in planta

Anaelle Baud * [1], Lucas Moriniere [1], Imane El Idrissi [1], Nicolas Taveau [2], Denis Costechareyre [2], Franck Bertolla† [1]

[1] Laboratoire d'Ecologie Microbienne, UMR CNRS 5557, UMR INRAE 1418, VetAgro Sup – Université Claude Bernard Lyon 1 – 16 rue Raphael Dubois, 69622 Villeurbanne, France
[2] GREENPHAGE SAS – GreenPhage – Clapier, France

The use of bacteriophages as biocontrol agents of phytopathogenic bacteria at different stages of the agricultural supply chain has shown promising results in recent years, notably on *Xanthomonas spp.* Bacterial leaf spot of lettuce caused by *Xanthomonas hortorum* pv. *vitians* (*Xhv*) is a major threat for lettuce producers worldwide due to the lack of effective disease control strategies. In order to explore the potential of phages to reduce the severity and incidence of this plant disease, we isolated and characterized several novel lytic phages. Their genome sequences, morphologies, growth kinetic parameters and host ranges were characterized. A transposon insertion sequencing experiment revealed that 36 genes predominantly involved in lipopolysaccharide biosynthesis were required for successful Φ-*Xhv1* infection of our *Xhv* model strain LM16734. Phenotypic analyses of transposon insertion and deletion mutants resistant to Φ-*Xhv1* suggested that this phage specifically binds lateral branches of the O-antigen to achieve its adsorption. Interestingly, some phage-resistant mutants defective in O-antigen biosynthesis showed a decreased fitness *in planta* and reduced motility on soft agar assays, resulting in a trade-off unlikely to occur *in planta*. Altogether, these results would pave the way to the design a phage cocktail combining various infection strategies and complementary host spectra, thus preventing the occurrence of resistances and ensuring the sustainability of the biocontrol.

Mots-Clés: Tn, seq, biocontrol, trade, off, Xanthomonas, phage resistance, LPS

*Intervenant
†Auteur correspondant: franck.bertolla@univ-lyon1.fr

Deciphering the interaction specificity of Clostridioides difficile bacteriophages

Alexia Royer * [1,2], Andrew Umansky [2], Robert Fagan [3], Olga Soutourina [1], Louis-Charles Fortier† [2]

[1] Université Paris-Saclay – Institute for Integrative Biology of the Cell (I2BC), Universitey Paris-Saclay, UniversitéParis-Sud, CNRS, CEA, 91400 Orsay, France – 3 rue Joliot Curie, Bâtiment Breguet 91190 Gif-sur-Yvette, France
[2] Université de Sherbrooke – 2500 boul. de l'Université, Sherbrooke, Québec J1K 2R1, Canada
[3] University of Sheffield [Sheffield] – Western Bank Sheffield S10 2TN, Royaume-Uni

Phage therapy is gaining attention as a promising solution against antibiotic resistance. Broad-spectrum antibiotics often result in microbiota disbalance due to off-target effects and contribute to issues such as *Clostridioides difficile* (*Cd*) infections with frequent relapses. *Cd* is a Gram-positive, spore forming bacterium, which is the leading cause of intestinal post-antibiotic nosocomial infection in industrialized countries. Phages, with their high host specificity, offer an alternative as a targeted therapy. However, a thorough understanding of *Cd*-phage interactions is crucial for successful infection control. Our research has revealed that the surface layer protein, SlpA, serves as a receptor for *Cd* phages. Moreover, we observed that different SlpA isoforms are involved in the specificity of interaction with them. We identified the D2 domain of the SlpA protein as important for infection by certain siphophages and myophages (Royer *et al*, Microbiol. Spectr. 2023). We are currently creating a collection of mutated SlpA isoforms to gain deeper insights into the regions of the protein that are involved in the interaction with various phages. Our work will also focus on the phage receptor binding proteins (RBP) known to interact with the bacterial host. Directed mutagenesis and interaction assays with purified RBPs using fluorescence microscopy will be done to refine our understanding of these interactions. Bioinformatics approaches are used to predict the RBP structures *in silico*, highlighting the structural diversity of these proteins. Ultimately, our findings will contribute to the design of recombinant phage cocktails targeting different SlpA isoforms to enhance the phage host range and pave the way for future applications of phage therapy against *Cd* infections.

Mots-Clés: Clostridioides difficile, phage receptor, phage host interactions, S layer, SlpA, bacteriophage therapy, bacteriophages

*Intervenant
†Auteur correspondant: Louis-Charles.Fortier@USherbrooke.ca

Molecular mechanisms of phage MDAΦ entry in Neisseria meningitidis

Clémence Mouville *† [1], Antoine Brizard [1], Julie Meyer [1], Anne Jamet [1], Hervé Lécuyer [1], Xavier Nassif [1], Mathieu Coureuil [1], Emmanuelle Bille‡ [1]

[1] Institut Necker Enfants-Malades – Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Université Paris Cité – Faculté de Médecine Necker 160, rue de Vaugirard 75015 Paris Cedex FRANCE, France

Neisseria meningitidis is a commensal bacterium of human nasopharyngeal mucosa that can cross 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. This phage can infect meningococci using their type IV pili (TFP) and hijacks the TFP secretin to exit without damaging the host. MDAΦ seems to increase the occurrence of diseases by increasing bacterial colonization at the site-of-entry. Our aim was to understand the molecular mechanism by which MDAΦ infects *N. meningitidis*. We focused on the first step of phage interaction with bacteria. Investigations with deletion mutants of genes involved in the TFP machinery have shown that phage entry requires a functional and retractable TFP. This result is consistent with the literature on Ff or CTXΦ phages that interact directly with the pili tip. However, we found no evidence here for the interaction of MDAΦ with the TFP tip. We therefore focused on the possible interaction between PilE, the main pilin forming the TFP fiber, and the phage capsid. We showed that the purified TFP and MDAΦ form bundles together. Since PilE is subjected to antigenic variation, we identified PilE variants associated with MDAΦ entry suggesting a direct interaction between PilE and phage particles Additional analysis of the charged amino acids of TFP and those of MDAΦ capsid coat supported our hypothesis. Finally, our results suggest that T4P whose electrostatic potential favors phage infection allow stronger adhesion of bacteria to endothelial cells compared to T4P variants that are less phage-infected. Taken together, our data support a new model of interaction between filamentous phages and type IV pili that could indirectly take part in the selection of pathogenic strains.

Mots-Clés: Neisseria meningitidis, filamentous bacteriophage, interaction, type IV pili

*Intervenant

†Auteur correspondant: clemence.mouville@gmail.com

‡Auteur correspondant: emmanuelle.bille@inserm.fr

Characterization of new molecular partners involved in filamentous phage infection.

Callypso Pellegri * [1], Laetitia Houot [1]

[1] Laboratoire d'ingénierie des systèmes macromoléculaires – Aix Marseille Université, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique – Batiment IBSM 31 Chemin Joseph Aiguier 13402 MARSEILLE CEDEX 20, France

Filamentous phages, a subclass of Inoviridae, are known to be non-lytic viruses. They are one of the simplest examples of viruses with a protein capsid protecting a circular single-stranded DNA genome. The persistent association they establish with the infected bacterium can profoundly affect the physiology or provide new pathogenic factors to the bacterial host. Filamentous phages are robust structure that requieres deep structural rearangements to " open " and tranfert their genome into the host cytoplasm. Studies conducted on M13, fd, and f1 coliphages identified bits and pieces of the E. coli infection process. However, the mechanisms involved in periplasm translocation and inner membrane perforation remain poorly understood. In our last publication, we identify new host partners involved in the phage translocation mechanism. Investigations using in vivo biochemical techniques show that the phage tip adhesion protein pIII interacts with TolQ and TolR, two proteins that form a conserved proton-dependent molecular motor within the inner membrane of the host cell. Furthermore, in vivo cysteine cross-linking studies show that the interactions between the pIII and TolQ or TolR occur between their transmembrane helical domains, possibly in response to the proton motive force status of the cell. These results allow us to propose a model for the late stage of filamentous phage translocation, which is mediated by multiple interactions with each individual component of the host TolQRA complex. (Pellegri et al., JBC 2023 ; doi: 10.1016/j.jbc.2023.105048).

Mots-Clés: structure, function relationship, protein, protein interaction, virus, host interaction, infection mechanism

*Intervenant

A novel strategy evolved by bacteriophage T5 to prevent E. coli general stress response

Nicolas Ginet * [1], Ambre Moreau [1], Paramita Sarkar [1], Ahlem Djedid [1], Aurélia Battesti [1], Mireille Ansaldi [1]

[1] Laboratoire de chimie bactérienne – Aix Marseille Université, Centre National de la Recherche Scientifique – 31 Chemin Joseph Aiguier 13402 MARSEILLE CEDEX 20, France

Bacteriophages and their hosts entertain complex relationships ranging from predation resulting in cell lysis to mutualistic benefits through lysogenic conversion. Coevolution during countless ages favored the emergence of various strategies on both sides to bypass each other's defense mechanisms. In bacteria, a wealth of new specialized anti-phage defense systems has been recently identified, adding up to the already famous Restriction-Modification (RM) and CRISPR-Cas systems. Conversely in bacteriophages, phage-encoded factors such as anti-RM or anti-CRISPR proteins enable phages to escape cellular defenses and the literature keeps reporting new viral factors targeting specific anti-phage defense systems.
Bacteria are primarily equipped with systems allowing the cell to cope with various stresses. The general stress response enables the cell to cope with all stresses, whatever the stress that originally induced it. It is triggered by different stresses such as entry into the stationary phase starvation or phage infection. It is usually regulated in bacteria by a specialized sigma factor, RpoS in the case of *Escherichia coli.* To prevent the onset of the general stress response that could interfere with the viral cycle, bacteriophages evolved proteins that interact with RpoS such as T7 protein Gp5.7 (direct binding to RpoS to prevent the host RNA polymerase to function) or T4 protein AsiA (direct binding to RpoS to redirect the host RNAP towards viral genes transcription).
We present here an entirely different strategy evolved by T5 to counteract RpoS functionality. We found out a pre-early small viral protein Gp00X produced during host takeover by T5 that alone is able to bring RpoS to proteolysis by the protease ClpXP in *E. coli.* We hypothesized that Gp00X by allowing RpoS proteolysis, prevents the establishment of the general stress response and favors the sigma70 factor instead of RpoS for viral genes transcription by the host RNAP.

Mots-Clés: Bacteriophage T5, host takeover, Escherichia coli, general stress response, defense systems

*Intervenant

Bacteriophage translocation across the intestinal barrier in inflammatory bowel disease

Clara Douadi * [1,2], Ilias Theodorou [1], Loïc Brot [1,2], Olivier Schiettekatte [1], Paolo Emidio Costantini [3], Roberto Saporetti [4], Alberto Danielli [3], Matteo Calvaresi [4], Quentin Lamy-Besnier [1,5], Marie-Agnès Petit [5], Harry Sokol [1,2], Véronique Carrière [1,2], Sophie Thenet [1,2,6], Luisa De Sordi† [1,2]

[1] Microbiote, intestin et inflammation [CRSA] – Centre de Recherche Saint-Antoine – Faculté de médecine Pierre et Marie Curie – Site Saint-Antoine – 6ème étage - 27, rue Chaligny – 75571 PARIS cedex 12, France

[2] Paris Center for Microbiome Medicine – Commissariat à l'énergie atomique et aux énergies alternatives, Institut National de la Santé et de la Recherche Médicale, CHU Pitié-Salpêtrière [AP-HP], Sorbonne Universite, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – Paris, France

[3] Dipartimento di Farmacia e Biotecnologie (FaBiT) – Alma Mater Studiorum - Università di Bologna, Bologna,, Italie

[4] Dipartimento di Chimica "Giacomo Ciamician" – Alma Mater Studiorum - Università di Bologna, Bologna, Italie

[5] MICrobiologie de l'ALImentation au Service de la Santé – AgroParisTech, Université Paris-Saclay, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – Domaine de Vilvert 78352 JOUY-EN-JOSAS CEDEX, France

[6] École pratique des hautes études – Université Paris sciences et lettres – 4-14 Rue Ferrus, 75014 Paris, France

Inflammatory bowel disease (IBD) is associated with gut barrier dysfunction, notably a hyperpermeability, and alterations of the gut microbiota, largely composed of bacteria and their bacteriophages (phages).
We investigated the interaction of phages with the intestinal barrier under basal and inflammatory conditions to test the hypothesis that increased gut permeability in IBD could lead to higher translocation of phages from the gut to the blood.

We applied purified phages 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. We tested phages of different morphologies: T4, M13 and ΦX174. The calcium chelator EGTA or pro-inflammatory cytokines were used to increase paracellular permeability.

In physiological conditions, 0.001 to 0.01% of the three phages crossed intestinal epithelial cells without inducing cytotoxicity, hyperpermeability or inflammation. Interestingly, phages could also translocate across intestinal tissues from mice. Barrier dysfunction significantly increased

*Intervenant

†Auteur correspondant: luisa.de_sordi@sorbonne-universite.fr

the translocation of ΦX174 as shown by a significant correlation with the paracellular permeability, but no difference was observed with the other phages. Using fluorescently-tagged M13 phages, we showed their adsorption and internalization within intestinal epithelial cells and human primary endothelial cells. Finally, the viromes of coupled faecal and blood samples from IBD and healthy humans revealed that most *Microviridae* present in the blood are also present in the faeces.

Our findings show that phages can cross the intestinal epithelial barrier, with an increased translocation rate for the *Microviridae* family when it is compromised, without triggering deleterious effects. They strengthen the hypothesis that phages can pass through the intestinal mucosa and reach the bloodstream with different crossing mechanisms. These results contribute to our understanding of phage dynamics in IBD and may have positive implications for the safety of phages as therapeutic agents.

Mots-Clés: bacteriophages, Microviridae, paracellular permeability, inflammation, IBD

Determining the main causes of phage resistance in clinical strains of Pseudomonas aeruginosa.

Clarisse Plantady *† [1,2], Axel Bernon [2], Rafael Gomes Von Borowski [2], Cindy Fevre [2], Gaëlle Demarre [2], Anne Chevallereau [1]

[1] Institut Cochin – Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Université Paris Cité – 24 rue du Faubourg Saint Jacques, 75014 Paris, France
[2] Phaxiam Therapeutics – Phaxiam Therapeutics – 60 avenue Rockefeller, 69008 Lyon, France

A current challenge for the development of phage therapy is to find the right phages targeting the strains responsible for a patient's infection. Consequently, much effort is made to constitute a broad portfolio of phages with different activity spectrum. A second concern is the emergence of phage resistance, which relies on mechanisms ranging from the blockage of phage adsorption to a variety of intracellular defenses. Bacteria can be intrinsically resistant to specific phages if they lack their receptors or if they carry defense systems that are active against them. When resistance does not pre-exist, it can rapidly evolve upon phage exposure.

Our objective is to determine the landscape of inherent resistance in a panel of 125 clinical strains of the opportunistic pathogen *Pseudomonas aeruginosa* associated with antibiotic treatment failure. A collection of 9 anti-*Pseudomonas aeruginosa* phages, belonging to 6 different genera and exhibiting receptor varieties, has been used to build an interaction matrix: a phage susceptibility test, which include a microdilution assay and a plaque assay, was performed on each strain to assess phage activity *in vitro*. When a strain was resistant to a phage, the underlying mechanisms were studied. First, we investigated whether phage resistance resulted from impaired adsorption and when this was not the case, the genomes of the strains were sequenced to identify potential intracellular mechanisms of phage resistance. We next aim to determine whether strains carrying multiple defense systems, or specific system combinations, have higher overall phage resistance.
These results allow to estimate the prevalence of intrinsic resistance, and its underlying causes, in clinically relevant isolates. They will contribute to improve the design of future phage discovery and cocktail assembly strategies and help to mitigate the impact of phage resistance on therapy outcomes.

Mots-Clés: phag, host interaction, resistance mechanisms, phagetherapy

*Intervenant
†Auteur correspondant: clarisse.plantady@etu.u-paris.fr

The blood virome in Crohn's disease

Quentin Lamy-Besnier * [1,2], Ilias Theodorou [1], Maud Billaud [1], Hao Zhang [1], Harry Sokol [3], Marianne De Paepe [1], Luisa De Sordi [2], Marie-Agnès Petit† [1]

[1] MICALIS – AgroParisTech, Université Paris-Saclay, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – Domaine de Vilvert 78352 JOUY-EN-JOSAS CEDEX, France
[2] Centre de Recherche Saint-Antoine – Institut National de la Santé et de la Recherche Médicale, Sorbonne Universite – 27 Rue Chaligny, 75012 Paris, France
[3] Centre de Recherche Saint-Antoine – Institut National de la Santé et de la Recherche Médicale, Sorbonne Universite, Assistance publique - Hôpitaux de Paris (AP-HP), Hôpital Saint-Antoine – 27 Rue Chaligny, 75012 Paris, France

Viral communities (viromes) are integral components of various body microbiomes. The intestinal virome has recently been associated with multiple diseases, including Crohn's disease (CD), suggesting implications for health. However, the virome of other environments, such as the blood, despite its potential as a disease biomarker and implications for health, remains poorly studied. Indeed, most studies of the blood virome have focused on eukaryotic viruses, and broad metagenomic investigations are lacking.
With the aim of improving our limited understanding of the link between CD and the microbiota, we conducted virome sequencing on 29 blood samples obtained from healthy individuals and CD patients. We rigorously addressed the specific limitations of this supposedly sterile environment (such as the low quantity of viruses, leading to high risks of studying contaminants) to provide the first comprehensive characterisation of the human blood virome.

We show that the blood virome contains diverse phages, which mostly infect Proteobacteria. Using fecal samples obtained conjointly from the same individuals, we also demonstrate that the contigs present in the blood virome are rarely found in the intestinal virome. We further explore other origins for the blood phages, including the oral environment. We also reveal that the blood virome is significantly different in CD patients compared to healthy individuals, contrary to the fecal virome which does not differ significantly between the two groups. Although very few phages were shared between the blood and intestinal environments, we found that CD patients had significantly more of them, suggesting that their altered intestinal permeability could lead to the passage of viral particles to the blood.
Collectively, these results unveil the presence of phages in human blood, suggest that their origin is only partially intestinal, and underscore differences in the blood virome composition between CD and healthy individuals.

Mots-Clés: blood virome, gut virome, Crohn's disease

*Intervenant
†Auteur correspondant: marie-agnes.petit@inrae.fr

Assessment of the carrier state life cycle in the lactic acid bacterium Oenococcus oeni following infection by distinct virulent phages

Amel Chaïb * [1], Florencia Oviedo Hernandez [1], Olivier Claisse [1], Claire Le Hénaff - Le Marrec† [1]

[1] Unité Mixte de Recherche Œnologie [Villenave d'Ornon] – Université de Bordeaux, Institut des Sciences de la Vigne et du Vin (ISVV), Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Bordeaux INP – 210 Chemin de Leysotte - CS50008 - 33882 Villenave d'Ornon, France

As part of our continuous efforts to characterize the interactions between virulent phages and the wine-associated lactic acid bacterium *O. oeni*, we designed different protocols to isolate Bacterial Insensitive Mutants (BIM) following challenges with the ex-temperate phage OE33PA, the virulent Krappator or Vinitor phages (1,2). As a final step, surviving bacteria were isolated twice on red grape juice agar. White colonies were selected since this phenotype was previously shown to indicate the absence of cell lysis (2). Cultures were prepared, tested for resistance to the challenged phages and stored at -20○C. Subsequent analysis confirmed the presence of BIM among these clones, but also revealed the presence of phages in others, accompanied by phenotypic modifications affecting parameters such as growth, phage sensitivity or CFU morphology. Remarkably, this was observed with the three phages but more frequently for Vinitor (3). Our results suggest the existence of a carrier state life cycle (CSLC) in *O. oeni*, possibly due to nutrient limitation and were further confirmed by sequencing. CSCL describes a population-level phenomenon, in which both sensitive and resistant bacteria exist alongside the phage. This cycle would maintain both bacteria and phage under unfavorable conditions, promoting horizontal gene transfer between phage and host (4). This particular interaction has been shown earlier in lactic *streptococci* (5,6). 1, Chaïb *et al.*, 2022 *Int J Food Microbiol.* 2022;383:109936 ; 2, Chaïb *et al.*, 2019, Appl Environ Microbiol. 85(19):e00997-19; 3, Philippe *et al.*, 2021, Front Microbiol. 2021;11:596541; 4, Cenens *et al.*, 2015, *PLOS Genetics* 11: e1005770; 5, Hunter, 1947, J. Hyg. (Cambridge), 45. 307-312; 6, Graham *et al.*, 1952. *J. Dairy Science*, 35:813-822.

Mots-Clés: Oenococcus oeni, Carrier state infection, BIM, CFU morphology

*Intervenant

†Auteur correspondant: clehenaff@enscbp.fr

TBD

Stefanie Barbirz * [1]

[1] MSB Medical School Berlin – Berlin, Allemagne

XX

Mots-Clés: Phage

*Intervenant

RNA-based defense systems in the interactions of Clostridioides difficile with phages

Olga Soutourina * [1]

[1] Institut de Biologie Intégrative de la Cellule – CEA, CNRS, Université Paris-Saclay – 1 avenue de la Terrasse, 91198 Gif/Yvette cedex, France

Clostridioides (Clostridium) difficile is the major cause of nosocomial infections associated with antibiotic therapy. The disruption of the colonic microbiota by antibiotics promotes colonization of the gut by *C. difficile.* Many aspects of *C. difficile* pathogenesis associated with adaptation strategies remain poorly understood. During the infection cycle, *C. difficile* survives in phage-rich gut communities by relying on defense systems like CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) system for adaptive prokaryotic immunity. Toxin-Antitoxin (TA) and abortive infection (Abi) systems also contribute to prophage maintenance, prevention of phage infection, and stress response. RNAs have emerged as key components of anti-phage defense systems in bacteria. CRISPR RNAs in complex with Cas proteins interfere with phage infection by targeting foreign nucleic acid for destruction. In type I TA (TITA), the antitoxin is a small antisense RNA that neutralizes toxin mRNA by inhibiting its translation and/or promoting its degradation and in type III TA, antitoxin RNA form a complex with the toxin leading to protein sequestration and neutralization. Prokaryotic defense systems cluster together forming defense islands and could be functionally linked. In *C. difficile*, RNA-based defense systems are frequently associated with prophages. We have demonstrated the defense function of CRISPR-Cas system characterized by unique features like the presence of many CRISPR arrays (8 on average), two conserved sets of *cas* genes (interference module alone or together with adaptation module), location of CRISPR arrays within prophages, frequently co-localized with TITA. We identified 13 TITA in *C. difficile* genome, 8 located inside prophages contributing to prophage maintenance. We are currently studying an Abi-like system involving noncoding RNA from the prophage of *C. difficile* hypervirulent strain. Genome-wide interactomics analysis suggested that RNA components of these defense systems could also have regulatory actions within global regulatory circuits important for pathogen fitness inside the host.

Mots-Clés: human pathogen, anti, phage defense systems, CRISPR, Cas, Toxin, Antitoxin, RNA

*Intervenant

Innate immunity: the bacterial connection

François Rousset *† [1]

[1] Weizmann Institute of Science [Rehovot, Israël] – 234 Herzl Street, Rehovot 7610001, Israël

Pathogens have fueled the diversification of intracellular defense strategies that collectively define cell-autonomous innate immunity. In bacteria, innate immunity is manifested by a broad arsenal of defense systems that provide protection against phages. The complexity of the bacterial immune repertoire has only been realized recently and is now suggesting that innate immunity has commonalities across the tree of life: many components of eukaryotic innate immunity are found in bacteria where they protect against phages. Here, I summarize recent findings on the conservation of innate immune pathways between prokaryotes and eukaryotes. I show that bacterial defense mechanisms can in turn catalyze the discovery of novel molecular players of eukaryotic innate immunity. We recently described ATP nucleosidases, immune effectors which cleave ATP molecules into adenine and ribose-5'-triphosphate during phage infection, thereby depriving phages of energy. Using phylogenetic analyses, we found that the immune ATP nucleosidase domain is found in a variety of eukaryotic organisms ranging from fungi to corals and insects, where it is embedded in a diverse set of proteins with a typical immune architecture. Taken together, our findings suggest that ATP degradation represents a novel mechanism of innate immunity that is conserved across the tree of life, highlighting the potential of bacterial defenses to expand our knowledge of eukaryotic immunity.

Mots-Clés: bacterial immunity, CBASS, ATP degradation, eukaryotic immunity

*Intervenant

†Auteur correspondant: francois.rousset@weizmann.ac.il

Molecular mechanisms of phage MDAΦ entry in Neisseria meningitidis

Clémence Mouville * [1], Antoine Brizard [1], Julie Meyer [1], Anne Jamet [1], Hervé Lécuyer [1], Xavier Nassif [1], Mathieu Coureuil [1], Emmanuelle Bille† [1]

[1] Institut Necker Enfants-Malades – Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Université Paris Cité – Faculté de Médecine Necker 160, rue de Vaugirard 75015 Paris Cedex FRANCE, France

Neisseria meningitidis is a commensal bacterium of human nasopharyngeal mucosa that can cross 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. This phage can infect meningococci using their type IV pili (TFP) and hijacks the TFP secretin to exit without damaging the host. MDAΦ seems to increase the occurrence of diseases by increasing bacterial colonization at the site-of-entry. Our aim was to understand the molecular mechanism by which MDAΦ infects *N. meningitidis*. We focused on the first step of phage interaction with bacteria. Investigations with deletion mutants of genes involved in the TFP machinery have shown that phage entry requires a functional and retractable TFP. This result is consistent with the literature on Ff or CTXΦ phages that interact directly with the pili tip. However, we found no evidence here for the interaction of MDAΦ with the TFP tip. We therefore focused on the possible interaction between PilE, the main pilin forming the TFP fiber, and the phage capsid. We showed that the purified TFP and MDAΦ form bundles together. Since PilE is subjected to antigenic variation, we identified PilE variants associated with MDAΦ entry suggesting a direct interaction between PilE and phage particles Additional analysis of the charged amino acids of TFP and those of MDAΦ capsid coat supported our hypothesis. Finally, our results suggest that T4P whose electrostatic potential favors phage infection allow stronger adhesion of bacteria to endothelial cells compared to T4P variants that are less phage-infected. Taken together, our data support a new model of interaction between filamentous phages and type IV pili that could indirectly take part in the selection of pathogenic strains.

Mots-Clés: Neisseria meningitidis, filamentous bacteriophage, interaction, type IV pili

*Intervenant

†Auteur correspondant: emmanuelle.bille@inserm.fr

Estimations of phage resistance and its impact on phage therapy against Pseudomonas aeruginosa respiratory infections

Ngoc Minh Chau Nguyen *† [1], Jacopo Marchi‡ [2], Sophia Zborowsky [1], Joshua Weitz [2], Laurent Debarbieux [1]

[1] Institut Pasteur [Paris] – Laurent Debarbieux – 25-28, rue du docteur Roux, 75724 Paris cedex 15, France

[2] Georgia Institute of Technology [Atlanta] – North Avenue, Atlanta, GA 30332, États-Unis

Antibiotic resistance represents a major public health challenge. One solution is the use of bacteriophages (phages) that target antibiotic-resistant pathogens. However, one of the major obstacles to phage therapy is the capacity of bacteria to develop phage resistance. We previously showed that the Pseudomonas aeruginosa strain PAK becomes resistant to phage PAK_P1 during the treatment of a pulmonary infection in immunodeficient mice, but not in immunocompetent mice. These results highlighted the synergistic antibacterial action of innate immune cells with phages in immunocompetent animals. In this study, we estimated the phage resistance rates of phages PAK_P1 as well as phage LUZ19, a phage belonging to another viral genus. Our results were compared to the Luria-Delbrück distribution, the expected outcome for the frequency of resistant mutants in a growing population in the case of random genetic mutation. We leveraged this model to compare the mutation rates of the phage PAK_P1 and LUZ19 through Bayesian inference. Next, we estimated the phage resistance rates of a mixture of phages PAK_P1 and LUZ19. Future experiments will be performed in increasingly complex environmental conditions with the addition of epithelial and innate immunity cells using a lung-on-chip system. We also explain how such information can be incorporated into eco-evolutionary models of interactions between phages, bacteria and the immune system in complex environments. In the long-turn, precise estimates of phage resistance rates in different environments will be pivotal to improve the design of phage cocktails and overall the efficacy of phage therapy.

Mots-Clés: Pseudomonas aeruginosa, phage resistance

*Intervenant

†Auteur correspondant: chau-ngoc-minh.nguyen@pasteur.fr

‡Auteur correspondant: jmarchi6@gatech.edu

Genetic screen to isolate bacterial mutants resistant to the toxicity of bacteriophage T5 genes

Godfred Morkeyile Annor * [1], Luiz Ramirez-Chamorro * [1], Pascale Boulanger * † [1], Ombeline Rossier * ‡ [1]

[1] Institute for Integrative Biology of the Cell (I2BC) – Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC) – 1 Avenue de la terrasse, 91198, Gif-sur-Yvette,, France

Bacteriophage T5 is a virulent phage that infects *Escherichia coli.* The T5 genome is composed of a linear dsDNA of about 121 kb. At the beginning of the infection, T5 expresses 17 so-called pre-early genes, responsible for the host takeover. Ectopic expression of individual viral genes under the control of an arabinose inducible promoter showed that seven pre-early genes (*A1, dmp, hegG, 08, 011, 013, 015*) were toxic to *E. coli* cells in the absence of other viral factors.To identify potential host targets and partners contributing to protein toxicity, a genetic screen was undertaken to isolate resistant mutants of *E. coli* and analyze their genome sequences. *E. coli* NEBalpha cells were transformed with plasmid pBAD24Cm carrying *A1*, grown overnight in the presence of glucose, and plated on a selective medium containing arabinose, yielding so far 65 independent resistant colonies for A1. Our primary interest was to identify bacteria with chromosome mutations, not plasmid mutations. Therefore, plasmids were extracted from the mutants and analyzed by electrophoresis to exclude mutants with detectable deletions or insertions. Plasmids extracted from 48 mutants had no visible change in plasmid size and were introduced into naïve cells, to test whether they carried mutations that abolished gene toxicity. In total, plasmids from three mutants (A1R22a, A1R22b, A1R28) were still conferring toxicity, suggesting possible mutations occurring in the *E. coli* chromosome. Genomic DNAs from the three resistant strains will be analyzed after whole genome sequencing. Our ongoing screening of resistant mutants for *A1* and other toxic pre-early genes will likely uncover bacterial functions targeted by phage T5 during host cell takeover.

Mots-Clés: T5 bacteriophage, Escherichia coli, host takeover, toxic genes, genetic screen

*Intervenant

†Auteur correspondant: pascale.boulanger-biard@i2bc.paris-saclay.fr

‡Auteur correspondant: ombeline.rossier@universite-paris-saclay.fr

Label free dynamic imaging: application to phage infected bacteria

Martine Boccara * [1], Viviane Ravet† [2], Claude Boccara [1]

[1] Institut Langevin - Ondes et Images (UMR7587) – Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, Sorbonne Universite, Centre National de la Recherche Scientifique – ESPCI, 1 Rue Jussieu 75238 Paris Cedex 05, France

[2] Laboratoire Microorganismes : Génome et Environnement – Centre National de la Recherche Scientifique, Université Clermont Auvergne – Campus Universitaire des Cézeaux, TSA 60026, 1 Impasse Amélie Murat, 63178 Aubière, France

Our aim is to develop live imaging tools and methods that are not destructive (fixation) and do not need molecular manipulations.
We first described a set up, which allows detection and analysis of phages (diameter from 35nm to 100nm and more) as well as other nanoparticles 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). We challenged our set up to compare it to labelling coupled flow cytometry of viruses and vesicles from different lakes from Massif Central.

We also recently developed a new tomographic approach (Optical transmission tomography) that has led to explore new fields: 1) We were interested to quantify metabolic activity within algae in different environmental conditions (b). We were able to detect and quantify movements within a cell under different environmental stresses.

2) We identified dynamic structures in *E coli*during infection by T5 phage (a generous gift of Pascale Boulanger)

All the results will be presented and discussed for more applications.

(a) Boccara, M., Fedala Y., Vénien-Bryan C., Bailly-Bechet M., Bowler C., Boccara A.C., Full field interferometry for counting and differentiating aquatic biotic nanoparticles: from laboratory to Tara Oceans. Biomed Opt Express Vol. 7, 3736-3746, (2016).
(b) Houda Bey, Florent Charton, Helena Cruz de Carvalho, Shun Liu, Richard G. Dorrell, Chris Bowler, Claude Boccara & Martine Boccara (2022): Dynamic Cell Imaging: application to the diatom Phaeodactylum tricornutum under environmental stresses, European Journal of Phycology, DOI: 10.1080/09670262.2022.2081732

Mots-Clés: membrane vesicles, microscopy, aquatic environment, viral factories

*Intervenant
†Auteur correspondant: viviane.ravet@uca.fr

DNA methylation levels and spontaneous mutations in E. coli lambda phage.

Magali Ventroux * [1], Marina Elez [1], Marianne De Paepe† [1]

[1] Département Microbiologie et Chaîne Alimentaire - INRAE – Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – INRAE Domaine de Vilvert F-78352 Jouy-en-Josas, France

During the cell cycle, replication errors can lead to spontaneous mutations, resulting in the appearance of genetic variants. Compared to their hosts, phages have much higher mutation and recombination rate than bacteria, even those that use their host's replication machinery. Thus, the estimated mutation rate of DNA viruses such as lambda phage is ~10-8 mutation/nucleotide/replication (m/n /r) i.e., ~ 100 times more than its host *E. coli.* The highly conserved DNA Mismatch Repair (MMR) mechanism corrects $>$ 90% of replication errors, significantly contributing to the avoidance of mutations. In *E. coli*, the key MMR proteins are the MutSLH complex, UvrD helicase, and Dam methyltransferase, which methylates newly replicated GATC sites. MutSLH uses the latency required for this methylation to recognize and cut the newly synthesized strand, triggering the repair. It has been shown that in replicative phages GATC sites are under-methylated, probably because the level of intracellular methylase Dam becomes limiting. We investigated whether lambda's high mutation frequency is due to inefficient MMR as a result of under-methylation of GATC sites. To this end, we characterized the effect of modulating Dam expression on lambda DNA methylation levels and mutant frequency. Our results show that Dam limitation may be involved in the high frequency of mutants in lambda, but that other mechanisms are also involved.

Mots-Clés: methylation, dam, mutations, lambda

*Intervenant

†Auteur correspondant: marianne.de-paepe@inrae.fr

Determining the scale and rates of adaptation of methane-oxidising bacteria in response to virus interaction in soil

Huaiyu Wang * [1], Sungeun Lee * † [2], Christina Hazard * ‡ [3], Graeme Nicol * § [4]

[1] École Centrale de Lyon – PhD student in Ecole centrale de Lyon – 36 avenue Guy de Collongue 69134 Ecully CEDEX, France
[2] École Centrale de Lyon – post doctorant – 36 avenue Guy de Collongue 69134 Ecully CEDEX, France
[3] École Centrale de Lyon – Research Leader – 36 avenue Guy de Collongue 69134 Ecully CEDEX, France
[4] Ecole centrale de Lyon – CNRS Director of Research – 36 avenue Guy de Collongue, France

Investigating virus-host interactions between populations in soil is challenging due to the vast diversity of both prokaryotes and viruses. The development of bioinformatic tools in conjunction with metagenomic approaches has enabled description of the diversity and complexity of virus communities in soil. However, characterising the dynamics and consequences of host-virus interaction remains challenging. We have developed the use of methane incubations for characterising methanotrophic bacteria and their viruses in a model system to examine interactions of native hosts and viruses in situ. This study is to determine whether active host-virus interaction can be identified in 'real-time' via incorporation of novel spacers in CRISPR arrays of methanotroph populations and to test the hypothesis that rates of change are proportional to rates of host activity. Soil microcosms were amended with a 10% methane headspace at 15 , 25 or 35 ◦C to produce different activity rates and destructively sampled in triplicate after 1, 2, 3, 4, 5, 6 and 12 weeks incubation for total and virome DNA isolation and sequencing. A parallel series of microcosms were also incubated with 13C to enable recovery of genomic DNA of growing hosts. Rates of methane oxidation varied with temperature (35 ◦C> 25 ◦C> 15 ◦C) while quantities of DNA extracted from virus fractions (via 0.2 μm filtration) differed with 25 ◦C> 35 ◦C> 15 ◦C. Amplicon sequencing of 16S rRNA genes revealed a significant increase in the relative abundance of Methylomonaceae at 15◦C and 25◦C, while Methylococcaceae were dominant at 35◦C. Metagenomic sequencing of 13C-enriched DNA allowed recovery of 148 metagenome-assembled genomes (MAGs) and 266 CRISPR arrays, with 29 found in contigs associated with MAGs including six methanotrophic populations. PCR assays are currently being developed for sequencing population-specific CRISPR arrays of methanotrophs. Novel spacer incorporation will then be compared to temporal virus community dynamics using viromes.

*Intervenant
†Auteur correspondant: sungeun.lee@ec-lyon.fr
‡Auteur correspondant: christina.hazard@ec-lyon.fr
§Auteur correspondant: graeme.nicol@ec-lyon.fr

Mots-Clés: methanotrophs, CRISPR, Methane, host, virus interaction, soil

Tackling Multidrug-Resistant E. coli: A Dual Strike Approach using a phage cocktail and a host-targeting molecule

Devon Conti * [1]

[1] Institut Pasteur [Paris] – Institut Pasteur de Paris – 25-28, rue du docteur Roux, 75724 Paris cedex 15, France

Devon CONTI1,3 (**PhD student**), Serena PETRACCHINI**2**, Marie-Anne NAHORI**2**, Céline MULET**1**, Amel METOUCCHI**2**, Emmanuel LEMICHEZ**2** and Laurent DEBARBIEUX**1**.

The multidrug resistant (MDR) *Escherichia coli* ST131 isolates, subclade *H*30Rx/C2, are dominant extraintestinal pathogenic *E. coli* (ExPEC) isolated from patients treated with fluoroquinolone and beta-lactam antibiotics 1,2. This heighted prevalence is likely due to an increased capacity to colonize the gut, form intestinal reservoirs, and perform invade tissues. The toxin CNF1 expressed by theses strains has been found to be a gut colonization factor involved in intestinal tissue invasion and promoting ST131 rising prevalence worldwide 3,4.The use of bacteriophages (phages) has been proposed as a treatment for reducing the gut colonization of enteric pathogens, without perturbing the host microbiota, as an alternative to antibiotics. However, bacteriophages are not expected to reach intracellular reservoir of E. coli ST131 strains, and will contribute to reduction of extracellularly present bacterial loads. However, the small piperidinamine-derived compound C910 was identified by our collaborators and found to block CNF1-mediated host cell invasion by the EC131GY strain, a representative of ST131 clones 5.

The aim of this project is to establish whether a double targeting of EC131GY with extracellular and intracellular weapons could lead to a synergistic efficacy in reducing both the intestinal reservoir and extracellular load of ST131*cnf+* strains.

So far, we have achieved the following:

- Phage screening and identification of optimal infection conditions *in vitro*
- Characterised ST131 gut colonisation of the gnotobiotic OMM12 mouse, including community and host response to infection.
- Determined *in vitro, ex vivo* and *in vivo* the efficacy of C910 and a phage cocktail in reducing intracellular and extracellular bacterial loads respectively.

*Intervenant

Our preliminary data strongly supports the *in vivo* evaluation of the anti-infectious effects of the piperidinamine-derived C910 compound in combination with the 3-phage cocktail.

Mots-Clés: multidrug resistant e. coli, in vivo, intestinal infections, ST131

Isolation and characterisation of bacteriophages targeting Klebsiella pneumoniae

Vincent Fontaine [1], Maria Sir Silmane [1], Sébastien Wurtzer [2], Laurent Moulin [2], Dominique Decré [1], Lisa Osbelt [3], Till Strowig [3], Alexandra Aubry [1,4], Catherine Eckert *† [1,5], Regis Tournebize * ‡ [1]

[1] Centre d'Immunologie et des Maladies Infectieuses – Institut National de la Santé et de la Recherche Médicale, Sorbonne Universite, Centre National de la Recherche Scientifique – 91 Boulevard de l'hôpital 75013 Paris, France

[2] Eau de Paris – Eau de Paris – Direction de la Recherche et du Développement pour la Qualité de l'Eau, RD Biologie. Ivry sur Seine, France, France

[3] Helmholtz Center for Infection Research – Department of Microbial Immune Regulation Inhoffenstr. 7 38124 Braunschweig, Allemagne

[4] Laboratoire de bactériologie - APHP – APHP, Pitié-Salpêtrière university hospital, Sorbonne Université UPMC Paris VI – Hôpital Pitié Salpétrière 89Bd de l'Hôpital 75013 Paris, France

[5] Laboratoire de bactériologie - APHP – APHP Groupe hospialier Est Parisien – Hôpital Saint-Antoine 184 Rue du Faubourg Saint-Antoine 75012 Paris, France

Klebsiella pneumoniae is a major nosocomial pathogen displaying one of the highest resistance rate to the main antibiotics among Enterobacterales. It is also considered a central hub for the emergence and the dissemination of antibiotics resistance within *Klebsiella* and Enterobacteriale species. It is therefore considered a critical pathogen requiring, with few other bacteria, the design of new and efficient therapies. One such promising strategy is based on the use of bacteriophages. To investigate the putative utilisation of bacteriophages to combat *Klebsiella pneumoniae* infection and colonisation, we isolated 54 bacteriophages from sewage water targeting 3 hypervirulent *K. pneumoniae* strains and 2 multi-drug resistant strains (ESBL and carbapenem-resistant). The phages were sequenced and evaluated for their capacity to infect a panel of clinical strains responsible of infections or isolated from the microbiota of healthy donors. We isolated phages with a narrow host range that were expressing a depolymerase and being able to infect strains expressing the same capsular type, and phages with a broader host range infecting strains of different capsule loci and sequence types. We also showed i*n vitro* that a cocktail of bacteriophage is able to prevent the growth of a ESBL *K. pneumoniae* and prevent the emergence of phage-resistance. This cocktail shows promising activity for potential evaluation and application *in vivo*.

Mots-Clés: Klebsiella pneumoniae, phages, MDR, hypervirulent strain

*Intervenant

†Auteur correspondant: catherine.eckert@aphp.fr

‡Auteur correspondant: regis.tournebize@inserm.fr

Development of virus-mediated nitrification inhibition

Felana Andrianjakarivony * [1], Rakshita Govind [1], Laura Sala-Comorera [2], Lorena Rodriguez-Rubio [2], Maria Teresa Muniesa Perez [2], Lisa Stein [3], Graeme Nicol [1], Christina Hazard [1]

[1] Environmental Microbial Genomics Group,Laboratoire Ampère, École Central de Lyon, – Ecole Centrale de Lyon, CNRS : UMR5005 – 36 Avenue Guy de Collongue, 69134 Ecully CEDEX, France
[2] Department de Genètica, Microbiologia i Estadística – Universitat de Barcelona, Diagonal 643, E-08028 Barcelona, Spain, Espagne
[3] Department of Biological Sciences – University of Alberta, Edmonton, AB, Canada, Canada

The impact of virus-host interactions on critical biogeochemical processes, such as nitrification, are largely unknown. Input of nitrogen (N) via fertilizers now exceeds that entering through natural processes with major environmental impact on terrestrial and aquatic systems. Existing strategies to increase N fertilizer use efficiency include inhibiting microorganisms that perform nitrification and transformation of fertilizer N with synthetic nitrification inhibitors. Our research will focus on understanding the diversity, activity and ecological impact of viruses infecting archaea and bacteria that have a central role in the process of nitrification. Our research will also evaluate the potential use of phage-based approaches as a targeted and natural control mechanism for reducing the activity of organisms contributing to nitrate leaching and N2O emissions. We employed two strategies for the cultivation of lytic viruses infecting soil ammonia oxidizers. The first is to use our curated culture collection of ammonia oxidizers, isolated from different environments, as hosts for viruses from soil. Our second approach is to isolate novel strains from soil and using them as potential hosts for viruses derived from the same location. This should enable us to determine if the application of cultivated nitrifier viruses represents a viable approach for decreasing soil N fluxes associated with fertilizer soil N transformation. If these objectives are successful, we would aim to subsequently develop the commercialization potential of this novel alternative form of biological nitrification inhibition.

Mots-Clés: fertilizer, nitrification, phage, ammonia oxidizers

*Intervenant

Characterization of RNA viruses from plant roots and rhizosphere soil

Yuanyuan Zhang * [1], Graeme Nicol† [1], Christina Hazard‡ [1]

[1] École Centrale de Lyon – Université de Lyon – 36 avenue Guy de Collongue - 69134 Ecully cedex, France

Soil contains a vast diversity of viruses that can infect prokaryote, fungal, animal and plant communities. However, in comparison to DNA viruses, the extent of RNA viral diversity in soil is less understood. To characterize RNA viral communities from plant roots and rhizosphere soil, we used samples taken from an agricultural field to compare different RNA extraction approaches, namely: total RNA from roots and soil, dsRNA from only roots, and RNA from prefiltered virus-like soil particles. This is followed by by metatranscriptomics and bioinformatics. Using the RNA-dependent RNA polymerase (RdRP) as a viral gene marker, RNA viruses were identified followed by analysis of community composition, phylogenetic diversity and potential hosts. A total of 3088 vOTUs were recovered, of which the largest proportion were from soil viromes followed by total RNA root samples. A total of 44 vOTUs were shared between plant root and rhizosphere soil. For Root samples, a greater viral diversity was found using total RNA extraction compared dsRNA. Plant viruses of the *Sobemoviridae* and *Virgaviridae* were most abundant in total RNA root and dsRNA root samples respectively, and insect-infecting *Solinviviridae* were also most abundant in total RNA root samples. Soil viromes had greater viral diversity in comparison to total RNA soil samples. Viruses of insect-infecting *Iflaviridae* were most abundant in soil viromes and fungi-infecting *Narnaviridae* in total soil RNA samples. Overall, RNA viral diversity, community composition and viral hosts found from different RNA extraction approaches and environmental sample type were strikingly different.

Mots-Clés: RNA virus, plant roots, rhizosphere soil

*Intervenant

†Auteur correspondant: graeme.nicol@ec-lyon.fr

‡Auteur correspondant: christina.hazard@ec-lyon.fr

Gram-negative Membrane Mimics and Other Tools to Study Host-Bacteriophage Interactions

Charles Arnaud *† [1,2], Hudson Pace [3,4], Marta Bally [3,4], Nina Broeker [1], Stefanie Barbirz‡ [2]

[1] Department of Medicine, Science Faculty, HMU Health and Medical University, Potsdam, Germany. – Olympischer Weg 1, 14471 Potsdam, Deutschland, Allemagne
[2] Department of Medicine, Science Faculty, MSB Medical School Berlin, Germany – Rüdesheimer Str. 50, 14197 Berlin, Deutschland, Allemagne
[3] Department of Clinical Microbiology, Faculty of Medicine, Umeå University, Umeå, Sweden – 6F och 6M, Universitetssjukhuset by 6 o 24 Umeå universitet, 901 85 Umeå, Suède
[4] Wallenberg Centre for Molecular Medicine, Umeå University, Umeå, Sweden – Umeå University 901 87 Umeå, Sweden, Suède

Caudoviridae phages infection cycle starts with docking itself to the host membrane and re-arranging parts of its tail into a structure allowing for safe channeling from the viral capsid to the host cytoplasm. To understand the mechanism of the initial infection steps at the host envelope we analyze bacteriophage genome transfer in vitro, employing O-antigen specific Salmonella model phages and various fluorescence techniques together with Gram-negative model membranes (Stephan, 2021; Broeker, 2019).
In this work, we analyzed Salmonella phage 9NA, a siphovirus with a long, non-contractile tail. Typically, DNA ejection from 9NA can be triggered when exposed to saturating concentration of its receptor using spectrofluorometry (Andres, 2012). Interestingly, we observed that unlike P22, a podophage having a closely related receptor binding protein but a different tail architecture (Podoviridae), kinetics of DNA ejection varied depending on lipopolysaccharides (LPS) preparation: If the LPS is presented to the virus as a pure component, DNA ejection was notably faster than upon presentation as part of purified outer membrane vesicles (OMVs). We conclude that membrane structure plays an essential role for triggering the phage molecular machine for DNA ejection. For example, the virus might "sense" the orientation and local densities of the LPS or the presence of proteins or membrane curvature in an OMV. This suggests a highly regulatory role of the membrane to mediate bacteriophage DNA ejection into the cell.
To further investigate these processes on the single molecule level, we have prepared supported lipid bilayers mimicking the Salmonella outer membrane and presenting the LPS receptor to monitor single phage DNA ejection by fluorescence microscopy (Mangenot, 2005). Moreover, on these membranes mimics we can study phage attachment in a more detailed manner via fluorescence microscopy and quartz crystal microbalance with dissipation monitoring (Peerboom, 2017).

*Intervenant
†Auteur correspondant: charles.arnaud@health-and-medical-university.de
‡Auteur correspondant: barbirz@uni-potsdam.de

Mots-Clés: Salmonella, 9NA, Siphovirus, LPS, in vitro model, Biophysique

Application en Thérapie et Biotechnologie

Optical trapping for the SUperfast Photonic detection of bacterioPhage Lysis (SUPPLY)

Nicolas Villa* [1], Enrico Tartari [1], Simon Glicenstein [2], Emmanuel Picard [2], Pierre R. Marcoux † [3], Marc Zelsmann [4], Gregory Resch [5], Emmanuel Hadji [2], Romuald Houdré [1]

[1] Institut de Physique, École Polytechnique Fédérale de Lausanne – CH-1015 Lausanne, Suisse
[2] Univ. Grenoble Alpes, CEA Grenoble, Grenoble INP, IRIG, PHELIQS, SiNaPS – Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA) - Grenoble – 38000 Grenoble, France
[3] Univ. Grenoble Alpes, CEA, Leti – Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA) - Grenoble – 38000 Grenoble, France
[4] University Grenoble Alpes, CNRS, CEA/LETI Minatec, Grenoble INP, LTM – Centre National de la Recherche Scientifique - CNRS – 17 Avenue des Martyrs, 38054 Grenoble Cedex 9, France
[5] Laboratory of bacteriophages and phage therapy, CRISP, Lausanne University Hospital (CHUV) – Pavillon 4 Office N°PAV4/02/207 Avenue de Beaumont CH-1011 Lausanne, Suisse

Introduction

The search for rapid methods in diagnostics, shorter than the lag time, yields a particular interest for methods applicable to very small biomasses (*e.g.* less than 1000 cfu). That is why single-cell characterization is a significant breakthrough compared to culture-based methods. In this context, integrated optical trapping devices on chips have the advantage of handling single micro-objects reversibly and with very low light power. Furthermore these devices enable both optical trapping and the simultaneous acquisition of information on the trapped object such as size, morphology and refractive index. This makes them very suitable for the study of biological objects, often more fragile and traditionally studied over large biomasses.

We present the optical trapping and non-destructive characterization, at the single-cell scale, of bacteria trapped on lineic microcavities or bidimensional photonic crystals. We aim at using these devices for both fast antibacterial or phage susceptibility testing at the single-cell scale.

Methods

The optofluidic device consists of a photonic chip covered by a fluidic chamber containing a suspension of the bacterial strain to be tested. An infrared laser is injected into the chip thanks to a first optical fiber. A second output fiber collects the transmitted laser light that passes through the chip. The optical trapping of biological objects can be monitored in two ways simultaneously : i) optical microscopy, from above, through the glass slide closing the fluidic chamber and ii) the amount of transmitted light collected by the output fiber.

Discussion

*Auteur correspondant: nicolas.villa@epfl.ch
†Intervenant

Various types of stresses impacting bacterial membrane have been investigated : thermal, osmotic, antibiotic and lytic phage. The membrane modifications of the trapped cell have been optically characterized for each of these stresses. The results evidence that on-chip devices for optical trapping may greatly enhance the characterization of bacteria at the single-cell scale, leading to fast antimicrobial susceptibility testing.

Mots-Clés: phage susceptibility testing, single cell characterization, optical trapping, membrane refractive index

Antigen self-anchoring onto bacteriophage T5 capsid-like particles for vaccine design

Pascale Boulanger *† [1], Nicolas Ducrot [1], Émeline Vernhes [1], Emmeline Huvelle [1], Ombeline Rossier [1], Malika Ouldali [1], Guy Schoehn [2], Linda Larbi Chérif [3], Clément Vanbergue [3], Karim Benihoud [3]

[1] Institute for Integrative Biology of the Cell (I2BC) – UMR 9198 Université Paris-Saclay, CNRS, CEA – 1 avenue de la Terrasse, 91198 Gif-sur-Yvette, France
[2] Institut de biologie structurale – Centre National de la Recherche Scientifique – Institut de Biologie Structurale, 71 avenue des martyrs, CS 10090, 38044 Grenoble Cedex 9, France
[3] Institut Gustave Roussy – UMR 9098 – 114, rue Édouard-Vaillant 94805 Villejuif Cedex -France, France

The rise in infectious diseases due to emerging pathogens is urging the development of new vaccines. Tailored virus-like particles attract growing interest for safe antigen delivery to the immune system. However their input to new vaccination strategies is limited due to the lack of universal virus-like scaffolds able to display large antigens through well-controlled coupling processes. While bacteriophages are recognized as promising vectors for antigen delivery, their use as vaccines in human clinical trials is confronted to regulatory issues. The design of new non-replicating particles derived from bacteriophages is needed to unlock the full potential of bacterial viruses as new vaccination platforms.
We have recently demonstrated the high potential of T5 capsid for new vaccine development. Taking advantage of the picomolar affinity of the decoration protein pb10 to T5 capsid, we designed pb10-antigen chimeras capable of strong self-anchoring onto T5 Capsid-Like Particles (CLP) devoid of viral DNA. We showed that mice immunization with T5 CLP displaying the model antigen ovalbumin elicit potent and long-lasting immune responses, with no need for extrinsic adjuvant. T5-derived CLP constitutes the first DNA-free bacteriophage capsid able to irreversibly display a regular array of 120 antigens through a highly efficient chemical-free grafting process.
Based on the engineering of pb10 chimeras, we propose a new platform for antigen delivery that is easy to produce in bacteria, cost-effective and highly stable in absence of cold chain. T5-CLP opens great prospects in the field of vaccination but also for various therapeutic and biotechnological applications.

Mots-Clés: bacteriophage T5, Capsid Like Particles, vaccine

*Intervenant
†Auteur correspondant: pascale.boulanger@i2bc.paris-saclay.fr

Engineered phages as a potential solution to treat Clostridioides difficile infections?

Louis-Charles Fortier *† [1], Andrew Umansky [1], Alexia Royer [1,2], Robert Fagan [3], Olga Soutourina [2]

[1] Université de Sherbrooke – Département de microbiologie et d'infectiologie Faculté de médecine et des sciences de la santé 3201 rue Jean Mignault Sherbrooke, Québec J1E 4K8, Canada
[2] Institut de Biologie Intégrative de la Cellule – Université Paris-Saclay – Bâtiment 21, 1 avenue de la Terrasse, 91198 Gif/Yvette cedex, France
[3] University of Sheffield – Molecular Microbiology, School of Biosciences, University of Sheffield, Sheffield, Royaume-Uni

For more than two decades, phage therapy has been suggested as a potential alternative to fight *Clostridioides difficile* (*Cd*) infections. However, despite some encouraging in vitro and in vivo results, currently available phages are not suited for therapeutic applications. The two main reasons explaining the inefficacy of currently available phages are that they are all temperate, meaning they can lead to phage resistance due to lysogeny, and most of them have a narrow host range. This prompted us to explore the possibility to genetically engineer *Cd* phages to overcome these limitations. Our group recently identified the surface layer protein SlpA as being a major phage receptor (Royer A. et al, Microbiol. Spectrum, 2023). We showed that different phages recognize and bind to one or more SlpA isoforms in a very specific manner. Using a combination of bioinformatics analyses, cryo-EM, AlphaFold predictions and functional assays, we identified the receptor-binding proteins (RBP) in several *Cd* phages, as well as putative CI repressors and integrase genes suspected to contribute to lysogeny. Our goal is to genetically engineer phages to remove the genes associated with lysogeny, and to improve their host range by engineering the RBP. As a proof of concept, we used a CRISPR-Cas strategy and genetically modified the phi027 prophage from the reference strain R20291 by replacing its RBP with the RBP from phage CD508. The recombinant phage phi027RBP508 acquired the host specificity of CD508, as demonstrated by infection and adsorption assays. We also deleted the CI repressor from phi027 and observed a lower frequency of lysogeny, but could not completely abolished it, suggesting that other genes will need to be removed. In summary, our recent advances suggest that it should be possible to create genetically engineered phages "à la carte" to target the most prevalent *Cd* isolates, while preventing lysogeny.

Mots-Clés: Clostridioides difficile, genetic engineering, CRISPR, Cas, lysogeny, host receptor

*Intervenant
†Auteur correspondant: Louis-Charles.Fortier@USherbrooke.ca

Selection of therapeutic anti-Staphylococcus phages: in-depth in vitro characterization and in silico study of putative bacterial determinants of phage activity

Camille Kolenda * [1,2], Mathieu Medina [1,2], Mélanie Bonhomme [1,2], Hugo Vaysset [3,4], Aude Bernheim [3], Leslie Blazere [1,2], Emilie Helluin [1,2], Patricia Martins-Simoes [1,2], Fabrice Pirot [5,6], Grégory Resch [7], Tristan Ferry [2,8], Frédéric Laurent [1,2]

[1] Service de bactériologie, Centre National de Référence des Staphylocoques, Institut des Agents Infectieux – Hospices Civils de Lyon – Lyon, France
[2] Equipe StaPath – CIRI, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Univ Lyon, F-69007, Lyon, France – Lyon, France
[3] Molecular Diversity of Microbes lab – Institut Pasteur de Paris – Paris, France
[4] AgroParisTech – INRA-AgroParisTech – Paris, France
[5] Plateforme FRIPHARM, Service pharmaceutique – Hospices Civils de Lyon – Lyon, France
[6] Laboratoire de Recherche et Développement de Pharmacie Galénique Industrielle – Université Claude Bernard - Lyon I (UCBL) – Lyon, France
[7] Center for Research and Innovation in Clinical Pharmaceutical Sciences (CRISP) – Lausanne, Suisse
[8] Department of Infectious Diseases – Hospices Civils de Lyon – Lyon, France

Phage therapy is promising to treat bacteria frequently multi-resistant to antibiotics, including *Staphylococcus aureus* (SA) and *Staphylococcus* non-*aureus* (SNA) species. The therapeutic potential of anti-*S. aureus* phages of various genera (*Kayvirus*, *Silviavirus* and *Rosenblumvirus*) remains to be extensively characterized and compared.
The activity of 20 anti-SA phages was evaluated against clinical isolates representative of the diversity of SA (n=30) and SNA (n=66) species. A representative set of 6 phages was selected for in-depth activity assessment combining solid- and liquid-based techniques against large panels of SA (n=150) and SNA isolates (n=66). An *in silico* approach was used to identify putative bacterial determinants of phage activity with a combination of tools: i) receptor typing (wall teichoic acids/WTAs encoding genes), ii) identification of systems which may alter phage infection after adsorption (DefenseFinder tool), iii) a genome wide associated study (GWAS) to identify loci statistically discriminating phage-susceptible and -resistant strains.

Host spectra depended on phage genus and were complementary. *Silviavirus* and *Rosenblumvirus* were the most active phages against SA but were specific of this species. *Kayvirus* exhibited a broader host range covering 4 to 6 SNA species. However, concordance rates between results of solid- and liquid-based techniques varied depending on phage and bacterial species. *In silico* analyses showed that WTA types had little or no effect on host range. DefenseFinder tool identified diverse types of putative anti-phage systems with a clonal distribution. Finally, a rational

*Intervenant

selection of genomic patterns identified by the GWAS revealed a limited number of candidate genes, including some detected by DefenseFinder, and some hosted by prophages.
The present study reports unprecedented *in vitro* characterization of a phage collection with high therapeutic potential and complementarity against *Staphylococcus* species. It provides new insights for phage susceptibility testing and identification of determinants of phage activity with a view towards therapeutic use.

Mots-Clés: Staphylococcus, phage therapy, activity, resistance, determinants

CRISPRi-seq identifies genes enhancing phage lysin Cpl-1 susceptibility in S. pneumoniae

Hugues De Villiers De La Noue* [1], Jonathan Save [1], Julien Denereaz [2], Xue Liu [3], Jan-Willem Veening [2], Gregory Resch †‡ [1]

[1] Laboratory of Bacteriophages and Phage Therapy, Center for Research and Innovation in Clinical Pharmaceutical Sciences (CRISP), Lausanne University Hospital (CHUV) – Pavillon 4, Avenue de Beaumont CH-1011 Lausanne, Suisse
[2] Department of Fundamental Microbiology, Faculty of Biology and Medicine, University of Lausanne – Biophore Building, Lausanne, Suisse
[3] Department of Pathogen Biology, International Cancer Center, Shenzhen University Medical School – 518060 Shenzhen, Chine

Streptococcus pneumoniae is a human pathogen killing above 1.6 million people per year. Moreover, antibiotic resistance remains a significant issue, which justifies the development of complementary strategies. Cpl-1 is a bacteriophage lytic protein (lysin) that has been shown to have high potential for clinical use. In this study, we aimed at using the novel CRISPRi-seq method to potentially identify genes interfering with Cpl-1 mechanism of action. Accordingly, we used an IPTG-inducible pooled CRISPRi composed of a 1928 sgRNAs library. Strain D39V was shotgun transformed with the library before being challenged with Cpl-1. Then, we compared the fitness of the sgRNAs to identify genes potentially involved in an increase of Cpl-1 susceptibility or tolerance. Identified genes were then further investigated using single CRISPRi clones or knockout mutants by kinetic growth assay. Eight genes were initially found to be linked to an increase in Cpl-1 susceptibility (*rodA, uppP, pbp2b, MreD, MreC, tacL, cps* and *mvk* genes) and seven to an increase in lysin tolerance (*DivIB*, *FtsE*, *FtsX, DivIVA*, *pgm*, *guaB* and a gene coding for a hypothetical protein). Further experiments confirmed the involvement of *rodA*, *uppP*, *pbp2B*, *MreD*, *MreC* and *mvk* genes as Cpl-1 susceptibility factors. However, no significant increase in Cpl-1 tolerance was observed for the candidate genes, which was not surprising given that natural selection of resistant clones has neither been reported in vitro nor in vivo. Although supplementary experiments are needed, we obtained promising preliminary results guiding the development of potential synergistic strategies for the treatment of pneumococcal infections.

Mots-Clés: CRISPRi, seq, S. pneumoniae, Lysine, Cpl1

*Auteur correspondant: hugues.de-villiers-de-la-noue@chuv.ch
†Intervenant
‡Auteur correspondant: gregory.resch@chuv.ch

Current and future clinical phage product quality control in Belgium.

Mathieu De Jode * [1], Marie Willocx [1], Céline Vanhee [1], Laure Cuignet [1], Jean-Paul Pirnay [2], Sarah Djebara [2], Pieter-Jan Ceyssens† [1]

[1] Sciensano – Rue Juliette Wytsman 14, 1050 Ixelles, Belgique
[2] Queen Astrid Military Hospital [Brussels] – 1120 Brussels, Belgique

As antibiotic resistance become a world health issue, new interest in phage therapy has risen all around the world. 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 1. This special status has made Belgium the most active country in the EU regarding phage therapy 2. This monograph states that clinical phage product (API) have to be prepared at the Queen Astride Military Hospital, and their quality controlled by Sciensano. This process starts with the construction of a genomic passport containing information regarding the phage's lifestyle, genome size and content. The bacterial production host's genome is also checked for the presence of active prophages and phage-inducible chromosomal islands. Secondly, each production lot is checked for microbial and prophage contamination, endotoxin levels, pH and titrated. The obtained results are condensed in a Certificate of Analysis which is returned to the manufacturer, and then transferred to the hospital pharmacy to enable preparation of the formulation upon a physician's prescription. To this day, this process has insured the quality and safety of more than 50 different phage lots, that were used to treat more than 100 patients. Overall, we will present this phage product quality control process in details, acknowledge its limitations and discuss its evolution in the future.

Mots-Clés: Phage, Therapy, Clinical, Production, Control, Reglementary

*Intervenant
†Auteur correspondant: Pieter-Jan.Ceyssens@sciensano.be

Anti-P. aeruginosa phage development for clinical applications

Cindy Fevre * [1], Clarisse Plantady [2], Antoine Guillon [3], Sandrine Le Guellec [4], Damien Piel [5], Nathalie Heuze-Vourc'h [6], Laurent Vecellio [6], Gaelle Demarre [1]

[1] Phaxiam Therapeutics – Phaxiam Therapeutics – 60 avenue Rockefeller, 69008 Lyon, France
[2] Phaxiam – Phaxiam Therapeutics – 60 avenue rockefeller, France
[3] Service de Médecine Intensive Réanimation, CHRU de Tours, Tours, France – CHRU Tours – xx, France
[4] DTF – DTFmédical – xx, France
[5] Phaxiam – Phaxiam Therapeutics – xx, France
[6] INSERM 1100 – CEPR – xx, France

Pseudomonas aeruginosa (PA) is the main cause of nosocomial lung infections, and the incidence of antibiotic-resistant strains is rising considerably. The clinical development of phage therapy in this indication is therefore particularly appropriate. A combination of 4 phages has been developed for specific administration by nebulization. In vitro models mimicking the mechanical ventilation system and exposure studies on non-human primates have shown that 5-10% of the phages loaded in the nebulizer reach the lungs. A single dose of the 4-phage combination nebulized in a mechanically ventilated pig model of acute pulmonary AP infection resulted in a 1.5-log decrease in bacterial load in the lungs compared with the untreated control within 24 hours. These data support the Phase IIb-III clinical trial of PyoPhaNEB to be launched in 2024. In parallel with clinical development the combination of 4 phages has been available for compassionate use since 2017. This allows us to collect 125 clinical strains associated with therapeutic failure. The analysis of the 4 therapeutic phages activity on this particularly relevant panel revealed first that the coverage of the 4-phage combination is declining slightly from 2022 and 2023. To better understand the underlying mechanisms of phage resistance, adsorption tests on Pa-resistant strains were carried out. Preliminary data suggests that resistance to Myoviridae phages tends to be associated with a lack of adsorption, in contrast to Podoviridae. In parallel genomic analysis of this panel strains was initiated in order to reveal the distribution of bacterial immune systems among resistant PA strains.

Mots-Clés: therapy, lung infections, clinical trial

*Intervenant

Phage therapy and multidrug-resistant Escherichia coli: Isolation, selection, production of therapeutic phages and demonstration of their in vivo effectiveness in gut decolonization

Floriane Laumay * [1], Guillaume Dalmasso [2], Racha Beyrouthy [3], Benjamine Lapras [4], Melanie Bonhomme [1], Remy Froissart [5], Mathieu Medina [1], Camille Kolenda [1], Tristan Ferry [6], Fabrice Pirot [4], Remy Bonnin [7], Laurent Dortet [7], Richard Bonnet [3], Frederic Laurent [1]

[1] Institut des Agents Infectieux [Lyon] – Hospices Civils de Lyon – Hôpital de la Croix-Rousse - 103 grande-rue de la Croix-Rousse - 69004 Lyon, France
[2] Equipe M2iSH, INSERM UMR 1071, INRAE USC 2018 – Institut National de la Santé et de la Recherche Médicale - INSERM, INRAE – Université Clermont Auvergne, France
[3] Centre National de Référence de la Résistance aux Antibiotiques [CHU Clermont-Ferrand] – CHU Clermont-Ferrand – CHU Clermont-Ferrand - Site Gabriel-Montpied, 58 Rue Montalembert, 63000 Clermont-Ferrand, France
[4] FRIPHARM – Hospices Civils de Lyon – Hospices Civils de Lyon, France
[5] Equipe Virostyle, UMR MIVEGEC 5290 UM-CNRS-IRD, Montpellier, France – CNRS : UMRUMRMIVEGEC 5290 UM-CNRS-IRD – Montpellier, France
[6] Service des Maladies Infectieuses et Tropicales – Hospices Civils de Lyon – Hospices Civils de Lyon, France
[7] Centre National de Référence Associé de la Résistance aux Antibiotiques [Hôpital Bicêtre AP-HP] – AP-HP Hôpital Bicêtre (Le Kremlin-Bicêtre) – 78 rue du Général Leclerc 94275 Le Kremlin-Bicêtre, France

Background

The global spread of *Escherichia coli* strains from the sequence type (ST) 131 is of particular concern due to their multidrug-resistant (MDR) profile. Phage therapy is an alternative to treat infection and/or eradicate colonization by these clones.

Thus, the objectives of the study were:

- To isolate anti-*E. coli* ST131 phages and to evaluate their activity on a large and representative panel of MDR ST131 clinical isolates;

- To evaluate the synergy between the most active phages;

- To evaluate the efficiency of the most promising phages to decolonize mice gut of a MDR ST131 clone.

*Intervenant

Methods

Eleven phages were isolated from wastewater, sequenced, and their spectrum of activity was determined by plaque assay on a large and representative collection of MDR ST131 clinical isolates (n = 190 from clades A, B, C1 or C2). The inhibition of bacterial growth by the 4 most active phages, alone or in combination, was then evaluated on 18 of the isolates in liquid medium. The phages with the strongest bactericidal activity were then produced, purified and used in a mouse model following the protocol of Galtier *et al.*, 2017.

Results

The phages with the broadest spectrum belonged to the *Tequatrovirus* (TQV) genus and preferentially targeted the clade A, while *Vectrevirus* (VCV) preferentially targeted the clade C2, suggesting a complementary activity to TQV. Consistently, TQV/VCV combinations significantly increased bacterial growth inhibition compared to phages alone. Additionally, a single oral dose of a specific TQV/VCV phage combination was sufficient to eradicate a carbapenem-resistant *E. coli* ST131 strain from the gut of mice. Further analyses indicated that the VCV-resistant mutants emerging both *in vitro* and *in vivo* became more susceptible to the TQV phage killing activity due to a better adsorption of the TQV phage on the wall of the mutant bacteria.

Mots-Clés: Bacteriophages, Phage therapy, Escherichia coli ST131, Multidrug, resistant, Phage combination, Phage activity synergy, Mice gut decolonization

Phages for Infections in Israel – The Israeli phage therapy center

Ran Nir-Paz * [1]

[1] IPTC and Department of Clinical Microbiology and Infectious Diseases, Hadassah-Hebrew University Medical center, Chair of the ESCMID-ESGNTA – Jerusalem Israel, Israël

Bacteriophages were introduced as a means to treat Infectious Diseases over 100 years ago. Interestingly, also in Mandatory Palestine, some preliminary work using bacteriophages was done lead by leaders in medicine and microbiology, however similarly to Western world, those were abandoned due to lack of well-established efficacy and the emergence of antibiotics. The new "modern" era was started almost 5 years ago, with a pivoting case in which a complex post-trauma patient was cured from 8 months of combined complex infection of both XDR *A. baumannii* and *K. pneumoniae* using a combination of 2 phages and 2 antibiotics. Following this case, we have established the Israeli Phage Therapy Center (IPTC), combining 2 forces from the Hebrew University (Ronen Hazan) and from the Hadassah Medical Center (myself). This joint operation has led to the creation of a few fundamental units within the center that enabled streamlining the treatment of more than 25 patients in various medical centers In Israel, and also the use of Israeli phages in other parts of the world with success rate of 70-86%. Currently, the IPTC Includes an active Israeli phage bank with more than 500 phages, a framework for Clinical Phage Microbiology (CPM), a clinical phage therapy unit and pharmacy services. Additionally using RedCAP tool, a CRF was created to standardize information about the treated cases and their CPM. Within the Israeli regulation we have created a pathway to provide phages to compassionate use cases. An Industry based clinical trial was conducted as well, and results should be available soon. Lastly, we are currently working on a unified protocol to further enable more compassionate treatments in a phase I type protocol to validate safety and tolerability of the use of phages in patients of need.

Mots-Clés: Phage therapy

*Intervenant

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

Sophia Zborowsky * [1], Jeremy Seurat† [2], Quentin Balacheff * ‡ [1], Emma Evrard [3], Joshua Weitz [4], Laurent Debarbieux§ [1]

[1] Institut Pasteur – Institut Pasteur de Paris – 75015 paris, France
[2] Georgia Tech – Georgia USA, États-Unis
[3] Institut Pasteur – Institut Pasteur de Paris – Paris, France
[4] Georgia Tech – Georgia, États-Unis

There is a renewed interest in bacteriophages - 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. 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: 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.

Mots-Clés: Bacteriophages, Antimicrobial resistance, Bacterial infection, Pneumonia, Macrophages, Neutrophils, phage therapy

*Intervenant
†Auteur correspondant: jeremy.seurat@gmail.com
‡Auteur correspondant: Quentin.Balacheff@pasteur.fr
§Auteur correspondant: Laurent.Debarbieux@pasteur.fr

Isolation and Characterization of New Bacteriophages Against Staphylococcal Clinical Isolates from Diabetic Foot Ulcers

Lucile Plumet *† [1], Madjid Morsli [2], Nour Mansour [1], Fernando Cavijo-Coppens [3], Laurence Berry [4], Albert Sotto [2], Jean-Philippe Lavigne [2], Denis Costechareyre *

[3], Virginie Molle * ‡ [1]

[1] Virulence Bactérienne et Infections Chroniques – Institut National de la Santé et de la Recherche Médicale, Université de Montpellier – Université de Montpellier, Place E. Bataillon, 34095 Montpellier, France
[2] Virulence Bactérienne et Infections Chroniques – Institut National de la Santé et de la Recherche Médicale, Université de Montpellier – Faculté de médecine, 186 chemin du carreau de lanes, CS83021, 30908 Nîmes Cedex 02, France
[3] Greenphage – GreenPhage – Cap Alpha, 34830 Clapiers, France
[4] Laboratory of Pathogen and Host Immunity – Centre National de la Recherche Scientifique, Université de Montpellier – Université de Montpellier, Place E. Bataillon,34095 Montpellier, France

Staphylococcus sp. is the most common bacterial genus in infections related to diabetic foot ulcer (DFU). The emergence of multidrug resistance bacteria represents a serious burden on public health systems. Phage therapy is an alternative treatment to antibiotics, overcoming the issue of antibiotic resistance. In this study, six phages (SAVM01 to SAVM06) were isolated from effluents and were used against a panel of staphylococcal clinical samples isolated from DFU. Genomic analysis revealed that the phages belonged to the *Herelleviridae* family with sequences similar to the *Kayvirus* genus. No lysogeny-associated genes, known virulence or drug resistance genes were identified in phage genomes. The phages displayed a strong lytic and antibiofilm activity against DFU clinical isolates, as well as opportunistic pathogenic coagulase-negative staphylococci. The results presented here suggest that these phages could be effective biocontrol agents against staphylococcal clinical isolates from DFU.

Mots-Clés: bacteriophages, biofilm, diabetic foot ulcer, Kayvirus, Staphylococcus sp.

*Intervenant
†Auteur correspondant: lucile.plumet@umontpellier.fr
‡Auteur correspondant: virginie.molle@umontpellier.fr

Caractérisation rapide de suspensions phagique par interférométrie. Partie 2 : vers la sécurisation en cours de process de purification.

Benjamine Lapras *† [1,2,3], Camille Merienne [1,2], Chloé Marchand [1,2], Alexia Von Kanel [1,2], Léa Usseglio [1], Camille Kolenda [2,4], Mathieu Medina [2,4], Frederic Laurent [2,4], Fabrice Pirot [1,2,3]

[1] Plateforme FRIPHARM, Hôpital Edouard Herriot, Hospices Civils de Lyon – n.a. – France
[2] Consortium PHAG-ONE - PHAGEinLYON – n.a. – N.A., France
[3] Laboratoire de Biologie Tissulaire et d'ingénierie Thérapeutique UMR 5305 – Université Claude Bernard Lyon 1, Centre National de la Recherche Scientifique – 7 Passage du Vercors 69367 Lyon cedex 07, France
[4] Centre International de Recherche en Infectiologie – Ecole Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, Université Jean Monnet - Saint-Etienne, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique – 21 avenue Tony Garnier 69365 Lyon Cedex 07, France

INTRODUCTION ET OBJECTIF :
Les suspensions phagiques doivent être purifiées pour répondre aux exigences réglementaires existantes et futures. La purification peut déstabiliser les phages, diminuant leur activité antibactérienne et donc leur efficacité clinique. En l'absence de technique rapide de titration, les risques de pertes de production par manque de maîtrise en cours de purification sont élevés.

Pour réduire ce risque, l'intérêt d'un *monitoring* rapide par microscopie à lumière interférométrique (ILM) en cours de purification est étudié.

MATÉRIELS ET MÉTHODES :

Un lot de lysat phagique anti-*Staphyloccocus aureus* (genre *Silviavirus*, morphotype myovirus) a été dilué au 10ème, lavé, concentré et formulé par filtration tangentielle (N=3). Un échantillon était prélevé à chaque étape pour mesurer : les concentrations et tailles médianes des particules par ILM (Vidéodrop, Myriade, France) (N=2) ; le titre par *spot-test* (N=3). L'écart entre la concentration particulaire moyenne (obtenue en quelques minutes) et le titre moyen (obtenu en 24 heures) était calculé ainsi (log(comptage) - log(titre))/log(titre).

RÉSULTATS :

La moyenne des écarts entre les deux mesures est de (5 ± 2) %. La taille des particules sus-

*Intervenant
†Auteur correspondant: benjamine.lapras@chu-lyon.fr

pendues est significativement réduite au cours de la purification ($p < 0{,}0001$, ANOVA) de (153 ± 2) nm à (146 ± 1) nm avec un titre moyen de suspension purifiée de (20 ± 2)E9 PFU/mL.

DISCUSSION ET CONCLUSION :

Si l'ILM ne doit pas remplacer la titration comme contrôle libératoire, elle permet tout de même d'obtenir en quelques minutes une estimation du titre phagique avec 10^(log(comptage)/(5 %+1)). S'il correspond aux spécifications et que la taille médiane des particules diminue, alors la purification est supposée efficace et la suspension peut être conditionnée.

REMERCIEMENTS :

Nous remercions la région AURA, l'ANR et la fondation HCL pour leurs financements, l'ensemble des membres du consortium PHAGE*in*LYON-PHAG-ONE, ainsi que la société Myriade pour le prêt d'un Vidéodrop.

RÉFERENCES :

doi: 10.3390/v13050939.
doi: 10.1002/jex2.75.

Mots-Clés: purification, quantification rapide, interférométrie

Rapid characterization of phage suspensions by interferometry. Part 1: A comparison of particle quantification to the reference method (titration).

Léa Usseglio * [1], Camille Merienne [1,2], Benjamine Lapras [1,2,3], Chloé Marchand [1,2], Mathieu Medina [2,4], Carole Paillet [1], Fréderic Laurent [2,4], Fabrice Pirot [1,2,3]

[1] FRIPHARM® - Pharmacie à usage intérieur, Groupement Hospitalier Centre - Hospices Civils de Lyon (HCL) – n.a. – n.a, France

[2] Consortium PHAG-ONE - PHAGEinLYON – n.a. – N.A., France

[3] Laboratoire de Biologie Tissulaire et d'ingénierie Thérapeutique UMR 5305 – Université Claude Bernard Lyon 1, Centre National de la Recherche Scientifique – 7 Passage du Vercors 69367 Lyon cedex 07, France

[4] Centre International de Recherche en Infectiologie – École Normale Supérieure [ENS] - Lyon, Université Claude Bernard - Lyon I, Université Jean Monnet - Saint-Etienne, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique - CNRS – 21 avenue Tony Garnier 69365 Lyon Cedex 07, France

Introduction:

Currently, a major setback for the expansion of phage therapy is the incompressible delay - minimum 1 day- to quantify the titer of active bacteriophages (BP) by spot test or double layer methods (PFU/mL).

Interferometric light microscopy (ILM) rapidly assesses (a few minutes) particle concentration in BP suspensions (particles/mL) and has the potential for routine quality control of injectable BP suspensions.

Objective:

This work compares the particle count obtained by ILM to the BP titer obtained by spot-test.

Materials and Methods:

Five dilutions of a purified BP suspension (*Silviavirus,* myovirus) from 3E8 to 3E9 PFU/mL were analysed (N=3) by ILM (Videodrop, Myriade) and by spot-test (reference method). The results - particle concentration (y) and phage titer (x) respectively - were compared with the following functions according to the NF EN ISO 15189:2012 guideline: f(xi) = (xi - yi) assessing relative residues, ensuring deviations stay within predefined limits and f(xi) = yi for linearity.

*Intervenant

Inaccuracy was assessed – results expressed in log - by dividing for each dilution the average measured concentration vs theoretical concentration (estimated by the dilution factor).

Results:

The linearity is suitable ($R^2 > 0.90$) between (i) theoretical versus measured particle concentration ($R^2=0.93$); (ii) and between the two methods (f(xi) = yi): R^2 of 0.92. For f(xi) = (xi-yi), 2 points are out-of-specification but close to the limits, resulting with a compliance rate of 89%. The averaged inaccuracy for all concentrations combined is of (5 ± 5) %.

Conclusion:
ILM method allows rapid (a few minutes) linear particle concentration assessment with an overestimation of phage titer of approximately 5 % inaccuracy. While it does not determine active phage concentration and therefore should not replace phage titer, ILM may be an interesting tool when rapid results are needed to estimate phage titer, e.g., before fill and finish, magistral preparation, etc.

Mots-Clés: interferometry, quantification

Characterization and genome analysis of novel bacteriophages vKpIN31 and vKpIN32, with lytic and anti-biofilm potential against Hospital acquired multidrug-resistant Klebsiella pneumoniae in Dakar, Senegal.

Issa Ndiaye * [1,2], Laurent Debarbieux [3], Guillaume Constantin De Magny [4,5], Abdoulaye Seck [2,6]

[1] Ecole doctorale Sciences de la Vie, de la Santé et de l'environnement, Université Cheikh Anta Diop – Dakar, Sénégal
[2] Pole de Microbiologie, Institut Pasteur de Dakar – BP 220, Dakar, Sénégal
[3] Laboratoire de Bactériophage, Bactérie, Hôte, Département de Microbiologie – Institut Pasteur de Paris – 75015 Paris, France
[4] Montpellier Ecology and Evolution of Disease Network – Université de Montpellier – Montpellier, France
[5] MIVEGEC – Centre de recherche IRD – BP 64501, 34394 Montpellier, France
[6] Faculté de Médecine, pharmacie et d'Odonto-stomatologie, Université Cheikh Anta Diop – Dakar, Sénégal

Klebsiella pneumoniae is a common gut colonizer of humans. However, it can be life-threatening when causing opportunistic infections, such as pneumonia, bacteremia, urinary tract infections, wound infections, meningitis, and neonatal sepsis. Moreover, the development of multidrug-resistant (MDR) isolates of *K. pneumoniae* leads to increase the severity and duration of infections in hospital settings, the so-called healthcare-associated infections.
This study aimed to isolate bacteriophages (phages) with high specificity, high lytic potential and antibiofilm activity against MDR *K. pneumonia*e strains isolated from Senegalese patients with hospital associated infections.

Two phages vKpIN31 and vKpIN32 were isolated and displayed a broad host range. Over the 28 strains displaying 20 different K locus types, vKpIN31and vKpIN32 were effective against respectively 12 and 9 strains with different K locus types. One-step growth curves showed that vKpIN31and vKpIN32 exhibited latent period respectively of 25 and 20 min. The average burst sizes were 5.6 103 and 3.2 103 PFU/cell, respectively. Both phages showed high thermal and pH stability. The lytic activity of the phages was evaluated in liquid medium at various multiplicities of infection (MOI 1, 10-1, 10-3). In all conditions the growth of their respective hosts was suppressed. Moreover, both phages displayed a characteristic anti-biofilm activity as observed by the reduction of adhered biomass in the 24-hour and 48-hour pre-formed biofilms.

The genome analysis indicated that both phages belong to the *Caudoviricetes* class, family *Demerecviridae*, subfamily *Sugarlandvirus* and genus *Sugarlandvirus* with estimated sizes of 77.115

*Intervenant

and 109.095 bp, respectively. No known genes associated with a temperate life cycle such as integrases, transposable elements, antibiotic resistance or bacterial virulence were detected in the genome of these phages.
Isolated vKpIN31 and vKpIN32 are proposed to be suitable candidates for phage therapy applications and could offer an effective solution for treating infections caused by MDR *K. pneumonia* strains.

Mots-Clés: Klebsiella pneumoniae, healthcare, associated infections, MDR, bacteriophages, phage therapy.

ENHANCING PHAGE THERAPY SAFETY: RELIABLE AND SENSITIVE PHAGE GENOME ANNOTATION WITH RTOOLS2 HIGH-THROUGHPUT PIPELINE

Antoine Culot * [1], Guillaume Abriat [1]

[1] Rime Bioinformatics – Rime Bioinformatics – 104 avenue du Général Leclerc, 91120, Palaiseau, FRANCE, France

Phage therapy is an exciting and promising approach to fight bacterial infections. However, ensuring the safety and efficiency of phages for therapeutic use requires a thorough understanding of their genomic properties. This process enables detection of genes that make phages potentially harmful for the subject of the therapy or the environment, such as antibiotic resistance, lysogeny, and virulence genes. Traditional bioinformatics tools designed for bacterial genomes are not well-suited for phage genomes due to their unique structure, leading to poor gene calling and function annotation. Recently, phage-focused tools have been released, such as Pharokka and rTOOLS2. rTOOLS2 is a multi-hypothesis, phage-focused annotation pipeline: its advanced algorithm uses the output produced by widely-used annotation tools to find more gene functions, with high evidence thresholds to avoid false positives. In this study, 135 phage genomes published in Genbank were annotated using Pharokka and rTOOLS2's high-thoughput version, and the results were compared. Pharokka was able to improve the average published annotation, as the average number of genes functionally annotated grew from 29.5% to 35.9%. On the other hand, rTOOLS2's high-throughput version was able to significantly increase the rate of annotated genes, reaching 54.6%. To promote the safest possible use of phages for patients and for the environment, it is key to use thoroughly characterized phages. rTOOLS2's high-throughput version can rapidly provide a strong basis for genome characterization. The use of curated databases ensure that meaningful annotations are provided, and results can be published with low risk of public database poisoning. Moreover, rTOOLS2 is able to produce more information, as it nearly doubled the number of annotated genes in the initially published genomes.

Mots-Clés: bioinformatics, annotation, phage therapy

*Intervenant

Phagothérapie : analyse des risques et gestion sécurisée des bactériophages à l'hôpital

Manon Mennecier *† [1], Claire Courtin [1], Marie Charlotte De Carne [2], Farahna Samdjee [1]

[1] Centre Hospitalier de Versailles André Mignot – pas de tutelle – 177 Rue de Versailles, 78150 Le Chesnay-Rocquencourt, France
[2] Centre Hospitalier de Versailles André Mignot – pas de tutelle – 177 Rue de Versailles, 78150 Le Chesnay-Rocquencourt, France

Face aux situations d'impasse thérapeutique, notre CRIOAC a souhaité étudier la faisabilité de la phagothérapie au sein de l'hôpital. La pharmacie à usage intérieur, rapidement sollicitée pour la gestion des bactériophages, a donc réalisé une analyse de risques a priori afin de sécuriser le circuit de ces nouveaux " médicaments ".
La méthode analyse globale des risques (AGR) est choisie. La création d'un groupe de travail permet la modélisation du système, la cartographie des dangers et l'identification des situations dangereuses (SD). Ces dernières sont classées par niveau de priorité (1, 2 ou 10). Les scénarii issus des priorités 1 sont cotés par criticité à l'aide d'échelles de vraisemblance/gravité. Des fiches de plans d'actions de réduction des risques sont rédigées pour les criticités C2 et C3. Une cotation résiduelle est ensuite effectuée afin d'engendrer un plan d'actions de contrôle des risques.

La cartographie a permis d'isoler 204 SD. Les principaux dangers identifiés concernent le facteur humain et le management, regroupant 52 SD. La phase la plus à risque est l'étape de préparation avec 59 SD identifiées. Pour les 84 SD de priorité 1, 109 scenarii de risques sont élaborés, 2 ont une criticité inacceptable (C3) et au total, 66 nécessitaient un plan d'actions. Dix-sept plans d'actions sont rédigés concernant notamment la formation des équipes à la manipulation des bactériophages et la gestion des dispositifs médicaux spécifiques. La seconde cotation a permis de réduire les criticités à 37 criticités C2 et de rédiger 4 plans d'actions de contrôle des risques avec indicateurs de suivi.
Cette étude a permis de révéler et d'anticiper les risques de cette nouvelle activité. La première application de la phagothérapie à un patient a permis de vérifier nos hypothèses. Le développement de cette activité à plus large échelle nous permettra de compléter cette cartographie avec davantage d'objectivité et d'exhaustivité.

Mots-Clés: phagothérapie, analyse de risque, pharmacotechnie, qualité

*Intervenant
†Auteur correspondant: manon.mennecier@gmail.com

TOPICAL DERMAL PLATFORM FOR THE CO-DELIVERY OF CURCUMIN AND STAPHYLOCOCCUS AUREUS PHAGES

Carole Armal * [1], Rosa Calderon-Jacinto [1], Kandas Traore [1], Jose Carlos Rodríguez Hernández [2], Ana Vallés Lluch [2], Damien Seyer [1], Guillermo Vilariño Feltrer [2], Agnès Mihajlovski [1], Emmanuel Pauthe [1], Violeta Rodriguez-Ruiz† [1]

[1] CY Cergy Paris Université - ERRMECe Laboratory, Biomaterials for Health Group – CY Cergy Paris Université - ERRMECe Laboratory, Biomaterials for Health Group – Rue Descartes, 95031 Neuville sur Oise, France

[2] Universitat Politècnica de Valencia (UPV), Centre for Biomaterials and Tissue Engineering (CBIT) – Camino de Vera s/n, PO Box 46022, Valencia, Espagne

The clinical evolution of some skin pathologies can be compromised by several factors including the presence of strong oxidative stress, microbial dysbiosis and infectious biofilms. The increasing prevalence of multidrug resistant pathogens and the high tolerance of biofilms to antibiotics make it necessary to search for therapeutic alternatives. In this regard, the use of lytic phages is a promising strategy to fight against infectious bacterial biofilms. In this work we propose a delivery platform containing Curcumin, a polyphenol compound from the rhizome of *Curcuma longa*, with anti-inflammatory and antioxidant activities, and *Staphylococcus aureus* phages as antimicrobial and anti-biofilm agents. The hydrophobic character and instability of Curcumin makes necessary its entrapment on specific vehicles. Nanostructured lipid carriers (NLCs), with excellent biocompatibility, represent a suitable option for the topical route. Both Curcumin-loaded NLCs and Staphylococcus aureus phages were entrapped into a hydrogel matrix that also provides moisturisation and helps for a topical application. Components of the delivery platform were prepared and studied as follows: Curcumin-loaded NLCs were produced by hot homogenization method and characterized by Dynamic Light Scattering (DLS) and Z-Potential, showing the feasibility in the production of stable, negatively charged Curcumin-loaded NLCs of 80 nm, (curcumin encapsulation efficiency of 85%). Staphylococcus aureusphages suspension of 1010 UFP/mL were produced and tittered by traditional top agar overlay method. Infectivity studies showed that the presence of Curcumin-loaded NLCs did not affect the lytic activity of Staphylococcus aureus phages. Preliminary cell viability studies of both Curcumin-loaded NLCs and Staphylococcus aureus phages were assessed on human Fibroblasts (BJ). First results show that developed NLCs were found to be non-toxic at concentrations below 0.54 mg/mL (containing 10 μM of curcumin). Preliminary results point that the combination strategy can represent a suitable option as topical skin treatment.

*Intervenant

†Auteur correspondant: violeta.rodriguez-ruiz@cyu.fr

Mots-Clés: Curcumin, Staphylococcus aureus phages, Nanostructured lipid carriers, hydrogel, topical skin treatment

Evaluation of the activity of combinations of anti-Staphylococcus aureus phages

Camille Kolenda * [1,2], Romain Garreau [3,4], Mateo Pouilly [1], Michael Burtin [4], Mathieu Medina [1,2], Tristan Ferry [2,5], Sylvain Goutelle [3,4], Frédéric Laurent [1,2]

[1] Service de bactériologie, Centre National de Référence des Staphylocoques, Institut des Agents Infectieux – Hospices Civils de Lyon – Lyon, France
[2] Equipe StaPath – CIRI, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Univ Lyon, F-69007, Lyon, France – Lyon, France
[3] Service de Pharmacie – Hospices Civils de Lyon – Lyon, France
[4] UMR CNRS 5558, Laboratoire de Biométrie et Biologie Evolutive – Université de Lyon, Université Lyon 1 – Villeurbanne, France
[5] Service de Maladies Infectieuses – Hospices Civils de Lyon – Lyon, France

The rationale for phage combination in personalised phage therapy compared to single phage treatments, including evaluation of synergistic effects between phages, is rarely detailed. In this study, we assessed the value of combining anti-*Staphylococcus aureus* (SA) phages by testing all possible intra- or inter-genus pairwise combinations of six phages from three different genera (*Kayvirus*, *Silviavirus* and *Rosenblumvirus*).
Bacterial growth inhibition by individual phages or combinations was tested in liquid medium against 25 SA strains at a multiplicity of infection of 1 over 48 hours by measuring the reduction in the area under the growth curve compared to untreated bacteria (Liquid Assay Score, LAS). The variation in activity between the phages used in combination or alone was calculated by: LAS of the combination - LAS of the monotherapy with maximal activity. Synergetic and antagonistic events were defined by LAS variations of +25% and -25%, respectively. For one of the strains for which synergy between 2 phages was observed, characterisation of resistant bacterial mutants arising in the presence of single phages or the combination was performed by comparing their genomes.

The mean activity variation for the 25 strains was between -6.6% and +6.0% depending on the phage combination. Among the 375 conditions tested (15 combinations*25 strains), 11 synergistic and 21 antagonistic events were observed. They were respectively more frequent for combinations of phages from different genera (*Rosenblumvirus-Silviavirus*) or from the same genus (*Silviavirus-Silviavirus*). Synergy between *Rosenblumvirus* and *Silviavirus* phages was associated with different mutations in resistant clones that emerged in the presence of the Rosenblumvirus phage (wall teichoic acid glycosylation gene *tarS*) or the combination (WTA backbone or peptidoglycan synthesis gene *tarO* or *femA*), whereas the *Silviavirus* phage alone had no activity.
Our study highlights a limited interest of combining anti-SA phages with rare synergistic events.

*Intervenant

Mots-Clés: phage therapy, Staphylococcus aureus, combination, synergy

Phage-mediated biocontrol against plant pathogenic bacteria: a model of collaborations between academics and applied research

Nicolas Taveau* [1], Hanane Amari [1], Laurène Robert [1], Marie Graindorge †‡ [1], Fernando Clavijo-Coppens [1], Denis Costechareyre † § [1]

[1] Greenphage – GreenPhage – Cap Alpha, 3 Avenue de l'Europe 34830 Clapiers, France

The capacity of agriculture to provide food to a growing world population is of crucial importance. Unfortunately, bacterial diseases of plants have continually reduced production since the advent of crop cultivation. Chemical agents have been used extensively to mitigate these losses. However, the rise of resistant bacteria, together with consumers' demand for organic products, presents problems that threaten sustainable agriculture. Through unique properties, bacteriophages offer a promising alternative for an effective biological control of plant pathogens. In this context, **GREENPHAGE** develops integrated phage-based strategies to control pathogenic bacteria intended for global health.
In collaboration with two academic partners, we investigate the pathosystem model lettuce/ *Xanthomonas hortorum* pv. *vitians* (Xhv). Bacterial leaf spot and headrot caused by Xhv places a major constraint on lettuce production. Currently, copper sulphate-based treatments used for Xhv early prevention has a strong environmental impact, and is often related to appearance of bacterial resistance/tolerance.

Our collaboration with the **Tropical and Mediterranean Symbioses Laboratory (Montpellier)** led to design innovative root inoculum for lettuce plants, combining the bio-fertilizing properties of arbuscular mycorrhizal fungi and bio-protective action of phages specific to Xhv. This project, called **SALAD CARE, was laureate of the grant funding "Companies on Campus" of Montpellier University.** Several bacteriophages were isolated and AMF/phage associations revealed a protective effect of AMF on phage survival.
With the **Microbial Ecology Laboratory of Lyon**, we recently started the **PHAG-2S project (laureate of CASDAR funding in 2022)** dedicated to develop phage-based solutions against Xhv. We isolated and characterized 22 virulent phages. *In vitro* experiments showed that one particular phage is able to infect all Xhv strains representing the diversity of the Xhv species. In addition, genome analyses demonstrate that ϕXhv1 and ϕXhv2 belongs to orphan *Straboviridae* phages. Phage cocktails assays will be performed soon *in planta* to evaluate their efficiency as biocontrol solution.

*Auteur correspondant: nicolas.taveau@greenphage.com
†Intervenant
‡Auteur correspondant: marie.graindorge@greenphage.com
§Auteur correspondant: denis.costechareyre@greenphage.com

Mots-Clés: Greenphage, academic collaboration, agriculture, Xanthomonas, lettuce, salad, plant pathogen, grant funding, phage therapy

Phage-mediated biocontrol in human health: a model of collaborations between academics and applied research

Marie Graindorge *† [1], Nicolas Taveau [1], Hanane Amari [1], Fernando Clavijo-Coppens [1], Denis Costechareyre * ‡ [1]

[1] Greenphage – GreenPhage – Cap Alpha, 3 Avenue de l'Europe 34830 Clapiers, France

Antimicrobial resistance (AMR) is a major problem and an enormous public health challenge. According to the WHO, AMR is one of the top ten global threats to public health. The projections are deeply concerning; AMR is responsible for approximately 1.3 million deaths worldwide annually, with an alarming possibility of escalating to ten million deaths by 2050. Whatever the infections, the exponential growth of resistance to various antimicrobial products adds to the gravity of the situation. The effectiveness of treatments for bacterial infections is becoming increasingly problematic, and the search for new antibiotics is facing a significant decline. Bacteriophages appear to be the most promising natural solution in this worrying situation. Through unique properties, bacteriophages offer an alternative for an effective and natural control of bacterial pathogens.
In collaboration with two academic partners, GREENPHAGE works on the development of innovative and sustainable phage-based therapy solutions for tackling superbugs and antimicrobial resistance. We investigate two typical infections exposed to the emergence of antimicrobial resistance. Our collaboration with the **Dr. Virginie Molle's team from the "Virulence Bactérienne et Infections Chroniques" laboratory (INSERM - University of Montpellier)** is dedicated to the isolation of bacteriophages active against *Staphylococcus spp.*, the most worrying bacterial genus in infections related to diabetic foot ulcer. A thesis, co-supervised by Greenphage and funded by the Ministry of Higher Education, Research and Innovation is in progress. This already led to the publication of a review and the submission of a research article. In parallel, since 2023, we work in concert with the **Department of Microbiology and Hospital Hygiene of CHU de Nîmes, led by Prof. Jean-Philippe Lavigne**, to develop bacteriophage-based solutions effective on uropathogenic *Escherichia coli* infections. Taken together, these two academic collaborations aim to develop bacteriophage-based solutions to prevent bacterial infections while performing scientific breakthroughs in phage science and therapy.

Mots-Clés: Greenphage, academic collaboration, human health, uropathogenic E. coli, Staphylococcus, antimicrobial resistance, diabetic foot ulcer infection, grant funding, phage therapy

*Intervenant
†Auteur correspondant: marie.graindorge@greenphage.com
‡Auteur correspondant: denis.costechareyre@greenphage.com

Developing the next generation of anti-staphylococci bacteriophage-based biomaterials

Emilie Cenraud * [1], Alice Schollhammer [1], Saeid Ekrami [1], Gregory Francius† [1], Erwan Andre [1], Fabienne Quiles [1], Xavier Bellanger‡ [1]

[1] Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement – Institut de Chimie du CNRS, Université de Lorraine, Centre National de la Recherche Scientifique – Université de Lorraine, 405 rue de Vandoeuvre, 54600 Villers-les-Nancy, France

Phage therapy is promising but there still are numerous gaps in knowledge about fundamental and practical aspects related to its implementation modalities. Through the ANR PHAGERIALS project, we aim to propose a prophylactic phage therapy approach, focused on human and animal skin infections caused by *Staphylococcus aureus* and *Staphylococcus pseudintermedius*. Hydrogels are safe and biocompatible materials already used as patches or sprayed for wound healing, or deposit on medical devices surface as coating. Our aim is to develop different hydrogels based on large polyions such as poly(allylamine), thus providing intrinsic antimicrobial activity, that will be loaded with phages for a synergistic bactericidal effect. Beyond using phages with a large host range, preliminary studies shown that phages suitable to be used in hydrogels must also present particular physical and chemical properties.
In this work, we present the first results obtained by using 8 model *S. aureus* phages from different families. We notably loaded phages by co-incubating them with pre-established multilayers hydrogels composed with alternative layers of hyaluronic acid and poly(allylamine). All but one of the phages seem to get into the hydrogel by passive diffusion at concentrations from 10^6 up to 10^8 PFU/mL of gel, corresponding to a 5 to 100 concentration factor by comparison to the phage suspension used.
We simultaneously characterized the hydrophobicity and charges of the phages. These phages properties are variable from one virus to another, some genetically close viruses having quite distinct properties, while some genetically distant viruses having similar ones. Among these properties, the phage negative charge, as well as the phage size/geometry, appear to be factors driving their behaviors in hydrogels. Replicates are needed but these results are promising for the development of efficient phages loaded biomaterials.

Mots-Clés: Phage therapy, Bacteriophage, Hydrogel, Staphylococcus aureus, Hydrophobicity, Charge

*Intervenant

†Auteur correspondant: gregory.francius@univ-lorraine.fr

‡Auteur correspondant: xavier.bellanger@univ-lorraine.fr

Engineering Staphylococcus aureus phage K genome using CRISPR-Cas9

Kandas Traore * [1], Julie Charles [1], Carole Armal [1], Violeta Rodriguez-Ruiz [1], Damien Seyer [1], Agnès Mihajlovski * † [1]

[1] Equipe de recherche sur les relations matrice extracellulaire-cellules – CY Cergy Paris Université – Maison Internationale de la Recherche, Laboratoire ERRMECe, Rue Descartes 95000 NEUVILLE SUR OISE Cedex, France

The increasing prevalence of multidrug resistant pathogens within the microbial world, in addition to the high tolerance of biofilms to antibiotics and common disinfectants, makes it urgent to develop new treatment methods for antibiotic-resistant bacteria and biofilms. Among the different alternatives to antibiotics currently being explored, phage therapy appears to be one of the most promising. The limitations inherent to phages, such as their host range restriction or their moderate antimicrobial and antibiofilm efficacy can be overcome thanks to recent advances in genetic engineering and synthetic biology. In this work, we intend to develop a CRISPR-Cas9 assisted engineering approach to allow the development of engineered *Staphylococcus aureus* phages. First, five crRNA targeting the Major Capside Protein (MCP) of *Staphylococcus aureus* phage K were designed using Geneious and assembled using Golden Gate assembly into the pCasSA vector. Chemically competent *Escherichia coli* DH10B were transformed with the recombinant vectors and Midiprep of each recombinant plasmid were prepared. Correctly assembled pCasSA vector with their respective crRNA were screened and confirmed *via* BsaI restriction. Then, *S. aureus* RN4220 were transformed with each construct using an innovative non-electroporation transformation method. This method allowed the transformation of *S. aureus* bacteria with an efficiency of 7.5 10^2 ± 1.5 10^2 UFC/108 viable cells. The efficiency of crRNA to cleave phage K genomes was determined by measuring the Efficiency Of Plating reduction (EOP). Among the five crRNA tested, three led to an EOP decrease comprised between two to three log. These first results demonstrate the possibility of implementing the CRISPR-Cas9 system inside *S. aureus* bacterial cells to target *S. aureus* phage genomes. We plan to couple this system with homologous recombination approaches as a tool to create innovative diagnostic and therapeutic *S. aureus* engineered bacteriophages.

Mots-Clés: Phage therapy, Biofilms, Staphylococcus aureus, CRISPR Cas9, Molecular engineering

*Intervenant

†Auteur correspondant: agnes.mihajlovski@cyu.fr

Liste des participants

- Alessio D'acapito
- Andrianjakarivony Felana
- Annor Godfred Morkeyile
- Baud Anaelle
- Benhamou Wakinyan
- Bille Emmanuelle
- Boccara Martine
- Boulanger Pascale
- Breyton Cécile
- Bröker Nina
- Chaïb Amel
- Chevallereau Anne
- Conti Devon
- Costechareyre Denis
- Croisier Delphine
- De Jode Mathieu
- De Sordi Luisa
- Debarbieux Laurent
- Decodts Maxime
- Douadi Clara
- Durand Hippolyte
- Durand-Dubief Michael
- Froissart Rémy
- Gaborieau Baptiste
- Gandon Sylvain

- Gendre Julia
- Henrot Caroline
- Huvelle Emmeline
- Laumay Floriane
- Laurent Frederic
- Le Dauphin Eve
- Marcoux Pierre R.
- Marion Saunier
- Maurin Amandine
- Mckeddie Cameron
- Mouville Clémence
- Mulet Celine
- Ndiaye Issa
- Ng Kamela
- Nguyen Ngoc Minh Chau
- Pellegri Callypso
- Petit Marie-Agnès
- Plantady Clarisse
- Quintens Johan
- Rey-Berthier Jérémy
- Rodriguez-Ruiz Violeta
- Rossier Ombeline
- Rouillon Christophe
- Rousset François
- Royer Alexia
- Sbardella Yanis
- Siby Heimrick
- Sieradzki Ella
- Soutourina Olga
- Tavares Paulo
- Vaysset Hugo
- Ventroux Magali

- Viala Julie
- Wielgoss Sébastien
- Zborowsky Sophia

Liste des auteurs

Abriat, Guillaume, 84
Alessio, D'Acapito, 27
AMARI, Hanane, 90, 92
ANDRE, Erwan, 93
Andrianjakarivony, Felana, 59
Annor, Godfred Morkeyile, 51
ANSALDI, Mireille, 40
ARMAL, Carole, 94
Armal, Carole, 86
Arnaud, Charles, 61
Arnaud, Charles-Adrien, 29
Aubry, Alexandra, 58

Balacheff, Quentin, 76
Bally, Marta, 61
Banfield, Jillian, 20
BARBIRZ, Stefanie, 46
Barbirz, Stefanie, 61
Bartoli, Julia, 33
Battesti, Aurélia, 40
BAUD, Anaelle, 36
Bellanger, Xavier, 93
Benhamou, Wakinyan, 13
Benihoud, Karim, 66
BENTANCOR, Leticia, 34
BERNHEIM, Aude, 4
Bernheim, Aude, 68
BERNIER, Juliette, 4
Bernon, Axel, 43
Berry, Laurence, 77
BERTOLLA, Franck, 36
Beyrouthy, Racha, 73
Billaud, Maud, 44
Bille, Emmanuelle, 38, 49
Bize, Ariane, 17
Blanquart, François, 13
Blazere, Leslie, 68
Blazewicz, Steven, 20
Boccara, Claude, 52
Boccara, Martine, 52
Bonhomme, Melanie, 73
Bonhomme, Mélanie, 68
Bonnet, Richard, 15, 73
Bonnin, Remy, 73
BOULANGER, Pascale, 66
Boulanger, Pascale, 51
Brasiles, Sandrine, 30
Breyton, Cécile, 27, 29
Brizard, Antoine, 38, 49
Broeker, Nina, 61
Bérard, Annette, 9

Calderon-Jacinto, Rosa, 86
Calvaresi, Matteo, 41
Cambillau, Christian, 28
Carrière, Véronique, 41
Cavijo-Coppens, Fernando, 77
CENRAUD, Emilie, 93
Ceyssens, Pieter-Jan, 71
Chaillou, Stéphane, 22
Chapleur, Olivier, 17
CHARACHON, Inès, 4
CHARLES, Julie, 94
Chaïb, Amel, 25, 45
Chevallereau, Anne, 43
Choisy, Marc, 13
Claisse, Olivier, 25, 45
CLAVIJO-COPPENS, Fernando, 90, 92
CLERMONT, Olivier, 4
Constantin de Magny, Guillaume, 82
Conti, Devon, 56
Costantini, Paolo Emidio, 41
COSTECHAREYRE, Denis, 36, 77, 90, 92
Coureuil, Mathieu, 38, 49
COURTIN, Claire, 85
COVES, Marion, 17
Cuignet, Laure, 71
Culot, Antoine, 84

Dalmasso, Guillaume, 73
Danielli, Alberto, 41
Dardalhon Beurrier, Elsa, 15
Darnault, Claudine, 27, 29
DE CARNE, Marie Charlotte, 85
De Jode, Mathieu, 71
De Paepe, Marianne, 6, 44, 53
De Sordi, Luisa, 8, 41, 44
De Villiers de la Noue, Hugues, 70
DEBARBIEUX, Laurent, 4, 11, 50
Debarbieux, Laurent, 76, 82
Decré, Dominique, 58
Demarre, Gaelle, 72
Demarre, Gaëlle, 43
DENAMUR, Erick, 4

Denereaz, Julien, 70
DEQUIDT, Tanguy, 4
DIB, Nicolas, 4
Djebara, Sarah, 71
Djedid, Ahlem, 40
Dortet, Laurent, 15, 73
Douadi, Clara, 41
Ducrot, Nicolas, 66
Dugat-Bony, Eric, 10
Dugat-Bony, Éric, 22
Dunne, Matthew, 31

Eckert, Catherine, 58
EKRAMI, Saeid, 93
EL IDRISSI, Imane, 36
Elez, Marina, 6, 53
Eugen, Pfeifer, 24
Evrard, Emma, 76

Fagan, Robert, 37, 67
Feltin, Chloé, 9
Ferry, Tristan, 68, 73, 88
Fevre, Cindy, 43, 72
Firestone, Mary, 20
Fontaine, Vincent, 58
Fortier, Louis-Charles, 7, 37, 67
Francius, Gregory, 93
Froissart, Remy, 73
Froissart, Rémy, 15

GABORIEAU, Baptiste, 4
Gaifas, Lorenzo, 27
Gandon, Sylvain, 13
Garreau, Romain, 88
Gendre, Julia, 22
GEORJON, Héloïse, 4
Ginet, Nicolas, 40
Glicenstein, Simon, 64
GOLDSTEIN, Jorge, 34
Gomes Von Borowski, Rafael, 43
Goulet, Adeline, 28
Goutelle, Sylvain, 88
Govind, Rakshita, 59
GRAINDORGE, Marie, 90, 92
Guillerm, Undina, 29
Guillon, Antoine, 72

Hadji, Emmanuel, 64
HAZARD, Christina, 54
Hazard, Christina, 12, 59, 60
Helluin, Emilie, 68
Henrot, Caroline, 11
HERSEN, Pascal, 4
Heuze-Vourc'h, Nathalie, 72
Houdré, Romuald, 64
Houot, Laetitia, 39
Huvelle, Emmeline, 66

Jamet, Anne, 38, 49
Jardillier, Ludwig, 17
Julian, Garneau, 9

Kolenda, Camille, 15, 68, 73, 78, 88

Lamy-Besnier, Quentin, 10, 41, 44
LANDAUD, Sophie, 22
Langlais, Alisa, 19
Lapras, Benjamine, 73, 78, 80
Larbi Chérif, Linda, 66
Laumay, Floriane, 15, 73
Laurent, Frederic, 73, 78
Laurent, Fréderic, 80
Laurent, Frédéric, 15, 68, 88
Lavigne, Jean-Philippe, 77
Le Guellec, Sandrine, 72
Le Hénaff - Le Marrec, Claire, 25, 45
LE MARREC, Claire, 22
LEE, Sungeun, 54
Lee, Sungeun, 12
Lindorff-Larsen, Kresten, 29
Liu, Xue, 70
Lopez, Julien, 6
Lossouarn, Julien, 10
Loux, Valentin, 6
Lukoyanova, Natalya, 30
Lécuyer, Hervé, 38, 49

MANSOUR, Nour, 77
Marchand, Chloé, 78, 80
MARCHI, Jacopo, 50
Marcoux, Pierre R., 64
Mariadassou, Mahendra, 6, 17
Martins-Simoes, Patricia, 68
Maurin, Amandine, 21
Mazéas, Laurent, 17
Medina, Mathieu, 15, 68, 73, 78, 80, 88
Mennecier, Manon, 85
Merienne, Camille, 78, 80
Meyer, Julie, 38, 49
Meynard-Doumenc, Coline, 8
Midoux, Cédric, 17
Mihajlovski, Agnès, 86, 94
MOLLE, Virginie, 77
Moreau, Ambre, 40
MORINIERE, Lucas, 36
Morris, Cindy, 9

Morsli, Madjid, 77
Moulin, Laurent, 58
Moura de Sousa, Jorge, 5
MOUVILLE, Clémence, 38, 49
Muniesa Perez, Maria Teresa, 59
MUNIESA, Maité, 34

Nassif, Xavier, 38, 49
Ndiaye, Issa, 82
Neumann, Emmanuelle, 27
NGUYEN, Ngoc Minh Chau, 50
NICOL, Graeme, 54, 59
Nicol, Graeme, 12, 60
Nicolas, Alexa, 20
Nir-Paz, Ran, 75

Orlov, Igor, 30
Orlova, Elena, 30
Osbelt, Lisa, 58
Ouldali, Malika, 66
Oviedo Hernandez, Florencia, 25, 45

Pace, Hudson, 61
Paillet, Carole, 80
Paillet, Thomas, 10
PALERMO, Marina, 34
Pauthe, Emmanuel, 86
Pellegri, Callypso, 39
PETIT, Marie-Agnès, 11, 34
Petit, Marie-Agnès, 10, 41, 44
Pett-Ridge, Jennifer, 20
Picard, Emmanuel, 64
Piel, Damien, 72
PINTO, Alipio, 34
Pirnay, Jean-Paul, 71
Pirot, Fabrice, 68, 73, 78, 80
Plantady, Clarisse, 43, 72
Plumet, Lucile, 77
Pouilly, Mateo, 88

Quaiser, Achim, 19
QUILES, Fabienne, 93
QUIROZ, Paulo, 34

RABOT, Sylvie, 34
Ramirez-Chamorro, Luiz, 51
Ravet, Viviane, 52
Resch, Gregory, 64, 70
Resch, Grégory, 68
RICARD, Jean-Damien, 4
RIVERO, Carla, 34
ROBERT, Laurène, 90
Rocha, Eduardo, 5, 24
ROCHE, Stéphane, 30
Rodriguez-Rubio, Lorena, 59
RODRIGUEZ-RUIZ, Violeta, 94
Rodriguez-Ruiz, Violeta, 86
Rodríguez Hernández, Jose Carlos, 86
Roose-Amsaleg, Céline, 19
Rossier, Ombeline, 51, 66
Rostøl, Jakob T, 14
ROUSSET, François, 48
Royer, Alexia, 37, 67
Rémi, Choquet, 13

Sala-Comorera, Laura, 59
SAMDJEE, Farahna, 85
Sanjuan, Rafael, 6
Saporetti, Roberto, 41
Sarkar, Paramita, 40
Saunier, Marion, 7
Save, Jonathan, 70
Schanda, Paul, 29
Schiettekatte, Olivier, 41
Schoehn, Guy, 27, 66
SCHOLLHAMMER, Alice, 93
Seck, Abdoulaye, 82
Seurat, Jeremy, 76
SEYER, Damien, 94
Seyer, Damien, 86
Sieradzki, Ella, 20
Sir Silmane, Maria, 58
Sokol, Harry, 41, 44
SOTTO, Albert, 77
Soutourina, Olga, 7, 37, 47, 67
Stein, Lisa, 59
Strowig, Till, 58

Taga, Michiko, 20
Tartari, Enrico, 64
Tavares, Paulo, 30
TAVEAU, Nicolas, 36, 90, 92
TESSON, Florian, 4
Thenet, Sophie, 41
Theodorou, Ilias, 41, 44
Torres-Barceló, Clara, 9
Tournebize, Regis, 58
TRAORE, Kandas, 94
Traore, Kandas, 86

Umansky, Andrew, 37, 67
Usseglio, Léa, 78, 80

Vallés Lluch, Ana, 86
Vanbergue, Clément, 66
Vaney, Marie-Christine, 30

Vanhee, Céline, 71
Vaysset, Hugo, 4, 68
Vecellio, Laurent, 72
Veening, Jan-Willem, 70
Ventroux, Magali, 53
Vernhes, Émeline, 66
VIALA, Julie, 33
Vilariño Feltrer, Guillermo, 86
Villa, Nicolas, 64
Vivès, Corinne, 29
Von Kanel, Alexia, 78

WANG, Huaiyu, 54
Wang, Yong, 29
WEITZ, Joshua, 50
Weitz, Joshua, 76
Willocx, Marie, 71
Wolfromm, Auguste, 8
Wurtzer, Sébastien, 58

ZBOROWSKY, Sophia, 50
Zborowsky, Sophia, 76
Zelsmann, Marc, 64
Zhang, Hao, 44
ZHANG, Yuanyuan, 60
ZINN-JUSTIN, Sophie, 30