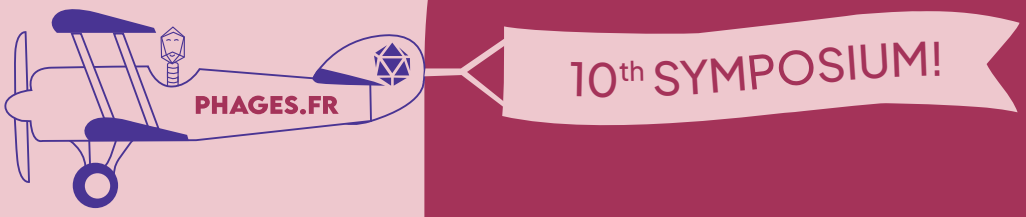
12-14 NOVEMBRE NANCY



ABSTRACT BOOK

2025 PHAGES IN NANCY

ORALES COMMUNICATIONS

2025 PHAGES IN NANCY

ECOLOGY AND EVOLUTION

POPULATION SCALE

Impact of infection by virulent phages on prophage induction in *Staphylococcus aureus*

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Staphylococcus aureus is a major human pathogen. By contributing to horizontal gene transfer and/or modulating the expression of virulence and resistance factors, both temperate and virulent phages are key drivers of *S. aureus* genetic remodeling and adaptation. However, the interaction between virulent phages, prophages and their bacterial host remains poorly understood in *S. aureus* despite the relevance of such knowledge for understanding the ecology, epidemiology, and pathophysiology of this major pathogen, as well as for therapeutic phage production and clinical application. This study aims to assess i) whether virulent phages can induce prophages and ii) whether the temperate phages co-produced with virulent phages can lysogenize other *S. aureus* strains.

The prophage content of 172 *S. aureus* strains was analyzed using PHASTER and BLAST. Forty-two strains with diverse prophage profiles (Sa1: n=10; Sa2: n=16; Sa3: n=16) were then infected at multiplicities of infection (MOI) of 10, 10^{-3}, or 10^{-5} with four different virulent phages from the Silviavirus, Kayvirus, or Rosenblumvirus genera. Prophage induction, resulting in temperate phage production, was assessed in various media using phage titration, PCR, and qPCR targeting virulence genes specific to each prophage type. Lysogeny events were evaluated by PCR targeting prophage genes and using the prophage-free *S. aureus* strain RN4220 as recipient strain.

Overall, our results indicate that under the conditions tested:

(i) exposure to virulent phages led to prophage induction, or enhanced induction in strains already capable of spontaneous induction, predominantly at an MOI of 10,

(ii) prophage induction phenomena appeared to be independent of the strain's genetic background, the type of prophage harbored, and the susceptibility of the strain to the virulent phage used,

(iii) an Eta/Sa1int-derived phage is capable of lysogenizing the RN4220 *S. aureus* strain, even in the presence of active lytic phages.

These results highlight the likely underestimated role of virulent phages in shaping the evolutionary dynamics of *S. aureus*, a major human pathogen. They also underscore the importance of evaluating prophage content in bacterial strains used for therapeutic phage production, and support the use of prophage-free strains for the safe and reliable bioproduction of therapeutic phages.

KEYWORDS: [Staphylococcus aureus, prophage, virulent phage, induction, lysogenization]

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tyPPing enhances identification of phage-plasmids

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Phage-plasmids (P-Ps) are mobile genetic elements that replicate as plasmids but can be induced and produce infectious virions as temperate phages. They often carry antibiotic resistance or virulence genes, making them important to study. However, their dual nature and genetic exchanges with phages and plasmids complicate their accurate detection (Pfeifer and Rocha, 2024).

Previous approach for P-P detection relied on outdated protein profiles combined with random-forest models, followed by complex clustering and manual curation. This strategy was time- and resource-consuming, limiting both efficiency and the ability to capture P-P diversity (Pfeifer et al., 2021).

To address these challenges, we developed tyPPing, a fast and user-friendly method for detecting and typing P-Ps in both complete and draft genomes. It targets the most prevalent, well-characterized P-P types, such as P1, N15, and SSU5, while allowing new types to be added as more sequences become available. tyPPing uses carefully constructed P-P type-specific signature profiles (based on conserved gene families) and combines two complementary strategies: (A) MinProteins, which checks for the presence of a minimum number of conserved genes, and (B) Composition, which analyzes gene patterns and organization of a target genome. Predictions are further refined using a genome size filter, and confidence levels are assigned to distinguish bona fide P-Ps from atypical cases.

We validated tyPPing on multiple databases, including RefSeq and collections of draft genomes, demonstrating both high speed and exceptional accuracy and sensitivity (>99%). tyPPing identified high-quality P-Ps in draft genomes, which we subsequently confirmed experimentally to be functional.

tyPPing outperforms existing approaches in sensitivity, efficiency, and scalability for well-characterized P-P types. However, the high diversity of P-Ps makes discovering new types challenging. Combining tyPPing with other methods, such as MM-GRC (Pfeifer et al., 2021), geNomad (Camargo et al., 2024), and vConTACT2 (Bin Jang et al., 2019), provides a robust framework for identifying both well-related P-Ps and more diverse or novel communities.

In conclusion, tyPPing represents a major advancement in P-P detection and systematic typing of prevalent types, while other methods remain valuable for detecting singletons or less studied P-Ps. tyPPing is openly available at https://github.com/EpfeiferNutri/Phage-plasmids.

KEYWORDS: phage-plasmids, phages, plasmids, genomics, mobile genetic elements

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Strain-specific phage predation modulates bacterial diversity under contrasting nutrient conditions

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Phages play a pivotal role in shaping microbial communities, but their ecological effects remain debated (Castledine & Buckling, 2024). The “kill the winner” hypothesis suggests phages regulate bacterial competition through competitive release, promoting microbial coexistence. However, evidence largely comes from ecological surveys documenting oscillations in phage and bacterial densities, without establishing causality. While such patterns could indeed result from top-down effects of phages on bacterial populations, it is also possible that the phages simply follow bacterial diversity which is dictated by bottom-up process, i.e. resource/nutrient availability. To establish such causality between viral-bacterial diversity, the simple and tractable gut microbiota of honeybees was studied combining ecological surveys with experiments. Paired viral and bacterial metagenomes from 49 individual bees were analyzed to identify phage-bacteria interaction networks and revealed correlations between viral and bacterial diversity. This work showed that viruses and bacteria form genetically and ecologically congruent modules. Within modules phages and bacteria showed strong correlations in composition across bees and diversity within individual bees. Strikingly, these correlations were particularly pronounced at the strain level, underscoring the importance of phage strain-specificity in natural systems (Ndiaye, M. et al., in press). An additional experiment using a synthetic community of 12 *Bifidobacterium* strains isolated from bee guts and isolated phages targeting all strains showed these phages displayed broad yet strain-specific host ranges, infecting multiple species but only particular strains within them, which were consistent with the metagenomic observation. Building on these findings, we passaged the 12 bacteria with and without phages, in either poor (sugar water) or diverse (pollen) nutrient environment. This full factorial design revealed a clear bottom-up effect on overall diversity, and a strong top-down effect on community composition. Interestingly we showed that in a poor nutrient environment, the kill the winner dynamics may explain transient increase in diversity although in diverse nutrient environment phage predation decreases the bacterial diversity. Taken together these results show that the top-down effect is stronger on the composition than the bottom-up. Finally, it seems that phages stabilize the communities over time. Our work highlights phages as key ecological players, with context-dependent effects on microbial diversity that possibly extend beyond the honeybee gut to host-associated microbiomes in general.

KEYWORDS: Host-associated microbiomes; competition; diversity; phages; honeybees.

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Microviridae diversity and host association in the Seine river

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Viruses are the most abundant biological entity on Earth, with an estimated 10^{27} particles in freshwater. Although viruses are often associated with pathogenicity, the majority of aquatic viruses are non-pathogenic bacteriophages, of which less than 1%have been identified until now. Understanding their diversity, dynamics and virus-host interactions is crucial for assessing their ecological roles in ecosystems such as the Seine River.

In this ongoing project, the viral nucleic acids were extracted and sequenced seasonally from both the inlet and outlet of Waste Water Treatment Plant (WWTP) as well as from the Seine River, using MGI sequencing technology. For each campaign (March, June, September 2024 and January 2025), about 1,8 billion sequencing reads were generated. The deep sequencing allowed the reconstruction of several thousands of predicted complete virus genomes. Most of these belonged to bacteriophages. For example, over 20,000 complete and unique genomes affiliated to the *Microviridae* family were reconstructed. Clustering and protein-sharing network analysis revealed around 2,000 different genera. Phylogenetic analysis of the concatenated capsid protein (VP1) and the DNA replication protein (VP4) combined with genome structure analysis allowed to explore the diversity of this family in the Seine River. Several new *Gokushovirinae*, a subgroup of *Microviridae* ubiquitous in the environment, were annotated and analyzed in detail. Further viromes and associated microbial metagenomes will be analyzed in order to investigate the viral dynamics as well as virus-host interactions in the water column of the Seine River upstream and downstream of Paris.

KEYWORDS: *Microviridae*, Seine River, Viral genomics, Bacteriophage

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The Phageome Of Apricot Trees And Its Association With Bacterial Canker Disease

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While phage roles in marine environments and the human gut are well studied, plant phageomes remain largely unexplored. Existing research mostly emphasizes phages as biocontrol agents, leaving ecological and epidemiological aspects understudied. *Pseudomonas syringae*, a diverse bacterial complex inhabiting multiple environments, includes pathogenic strains responsible for apricot tree canker, a disease that damages twigs and can kill young trees. However, the diversity and role of phages in this pathosystem remain to be elucidated.

This study aimed to characterize the apricot tree phageome and compare healthy versus diseased tissues. We purified viral particles and applied viral metagenomics to profile phage communities across soil, buds, and twigs. In parallel, metabarcoding was used to assess *P. syringae* diversity. Bacterial analyses revealed a distinct diseased-associated *P. syringae* signature in symptomatic twigs absent from other substrates. We identified 12,547 phage fragments (vOTUs), 28% of which had predicted hosts. Phage richness was highest in soil and comparable in the phyllosphere (buds and twigs). Each niche harbored distinct phage populations, though some were ubiquitous across trees. Diseased twigs notably lacked abundant phages infecting Actinomycetota, Pseudomonadota, and Bacillota bacterial phyla, while showing an increased relative abundance of *Pseudomonas* phages.

These results suggest a potential phage community dysbiosis associated with canker disease. The rise of *Pseudomonas* phages may reflect a response to bacterial proliferation in infected tissues, while the loss of Actinomycetota, Pseudomonadota and Bacillota phages indicates disruption of the normal phage–bacteria equilibrium. The present study represents a pioneering investigation of the phageome associated to plant health. Understanding phage ecology in agriculture offers new insights into phytopathogen dynamics, with potential applications for biocontrol and disease monitoring.

KEYWORDS: phageome, metagenomic, ecology, *Pseudomonas syringae*, apricot

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Latent Period Variability and Viral Dynamics

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Bacteriophages (phages) are fascinating organisms—tiny, abundant, and profoundly influential in microbial ecosystems. A critical aspect of understanding phage ecology lies in their life-history traits, which describe how they infect and replicate. These traits not only influence the phage's ability to reproduce and spread but also shape their interactions with bacterial hosts, affecting population dynamics and evolutionary processes. While life-history traits are often studied as fixed parameters, phages exhibit significant variability in these traits. Here, we explore the nature and consequences of such variability, focusing on latent period variability through a combination of theoretical modeling and experimental approaches. Using a non-linear dynamical model that accounts for latent period variability during lytic infection, we demonstrate that inherent variability in the latent period leads to systematic underestimation of the latent period mean when using standard methods of viral trait characterization, such as the one-step growth curve. To address this issue, we build a computational framework that accurately characterizes viral life-history traits, including latent period variability, from time series of viral densities that capture multiple rounds of infection. We apply this computational framework to experimental data of *Synechococcus*-infecting phage Syn9 and predict the latent period distribution of Syn9 by fitting experimental population-scale time series. We then develop a single-cell lysis detection protocol that enables characterization of latent period distributions at the individual cell level. Our results show that the predictions made using the population-scale computational framework align with the single-cell data, providing evidence that heterogeneity at the single-cell scale can be characterized using population-level data. Lastly, we infer the relationship between latent period and burst size at the single-cell level, revealing a one-piece linear relationship and demonstrating that variability in the latent period drives the observed variability in burst size. Overall, we demonstrate that variability in life-history traits is a fundamental driver of viral dynamics and offer a robust framework for quantifying and interpreting that variability in both theoretical and empirical contexts.

KEYWORDS: life-history traits ; single-cell ; heterogeneity ; computational modeling ; viral dynamics

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PHAGE-HOST INTERACTION

CELLULAR SCALE

Diversity and Activity of Prophages in *Streptococcus agalactiae*: Insights into Phage–Host Interactions

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Streptococcus agalactiae, or group B *Streptococcus* (GBS), is an opportunistic pathogen present in 10 to 30% of the human population. It is the leading cause of neonatal sepsis and meningitis, responsible for approximately 90.000 deaths annually worldwide. GBS is also increasingly implicated in infections of immunocompromised adults (Seale A.C. *et al*, 2017). Prophages and their genetic cargo are susceptible to confer selective advantages to their bacterial host, and they are widespread and diverse within the species, with 19 major groups described, representing on average 5% of the bacterial chromosome (Kovacec V., *et al.*, 2024). Despite their potential interest for the medical field, and even though they are well inventoried at the genomic level, GBS prophages remain poorly characterized, particularly in terms of their physiological behavior.

In our project, we investigated a panel of 12 GBS strains representative of the most epidemiologically relevant genotypes, harboring 23 distinct prophages, including Prophage-Inducible genomiC Islands (PICIs), a type of phage satellite. In parallel, phage resistance mechanisms were investigated using two recently developed bioinformatics tools, revealing a wide array of bacterial defenses suggesting frequent phage-host interactions.

All prophages, including PICIs, were found to be inducible under specific conditions. However, while induction was relatively straightforward, propagating these phages in a lytic manner to other strains proved very challenging. Interestingly, lytic activity on the target GBS strains could be visualized as lysis plaques on bacterial lawns only after propagating first the induced prophages on an intermediate (propagation) GBS strain, further complicating the network of interactions between these phages and their host bacteria. A glimpse of these interactions will be presented and discussed, underscoring the complex and dynamic interplay between GBS and their prophages, revealing both the challenges of phage propagation and the intricate network of phage-host interactions that shape GBS physiology.

KEYWORDS: Prophages, genomic diversity, lysis-lysogeny balance, phage propagation

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From Host to Phage: evolution and function of Nucleoid-Associated Proteins

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The evolution of bacteriophage genomes is shaped by co-evolution with bacterial hosts, often as part of an evolutionary arms race: phages aim to complete their infection cycle, while bacteria deploy defence systems to block them. A key challenge in this conflict is protecting genome integrity: phages must shield their DNA from host defences and access the host genome during lysogeny. Nucleoid-associated proteins (NAPs), which bind, protect, and organize DNA in bacteria, may thus play a central role in phage-host conflicts. NAPs are diverse in their binding behaviour and influence all DNA-based processes, including transcription, replication, and DNA repair. In several cases, phages have co-opted host NAPs for crucial steps in their life cycle such as integration or replication: Lambda depends on *Escherichia coli*'s IHF for integration (Miller and Friedman, 1980), and Fionnbharth phage hijacks its host's Lsr2 to organize its viral factory (Dulberger *et al.,* 2023). Sometimes, phages also encode their own NAP homologs, likely acquired from bacteria and adapted to their own purpose. The TF1 protein from SPO1, for instance, is a homolog of the widespread bacterial NAP HU (Greene *et al.,* 1984). But are NAPs a common part of phage toolkits? Using bioinformatic tools, we surveyed 168,201 diverse phage genomes for the presence of homologs of 13 bacterial NAPs. We find that most NAPs are rare in phage. However, four - HU, Hc1 and the xenogeneic silencers Lsr2 and H-NS - are common constituents of phage genomes, and we assess their role in phage biology by characterizing the phylogenetic distribution of NAP-bearing phages, their lifestyles, genomic features, and co-variance patterns with other proteins. We conclude that NAPs represent common but poorly understood constituents of phage genomes, highlighting chromatin as a frequent battleground in phage-host co-evolution.

KEYWORDS: NAPs; chromatin; evolution; arms race

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Prophage-inducing bacteriocins are frequent in *Escherichia coli* intestinal isolates

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In the gut microbiota, lysogens - bacteria carrying prophages - are particularly frequent and prophage induction is widespread, as indicated by the abundance of temperate phage reads in human virome data. Intriguingly, some prophages are induced in the gut but not *in vitro*, pointing to host or environment-specific cues involved in their induction. However, the relevant inducers remain poorly defined, as most investigations have relied on *in vitro* assays lacking an ecologically relevant environment including various microbes.
Besides, bacterial competition for resources is highly important within the gut microbiota and involves the production of antibacterial compounds, including bacteriocins, that are ribosomally synthesized antimicrobials displaying typically a narrow killing spectrum and especially abundant in Enterobacteriaceae. Importantly, several bacteriocins can activate the SOS-response regulator RecA, a key player in the canonical prophage-induction pathway.

In this study, we investigated the interplay between bacteriocin-producing strains and lysogens. We performed a phenotypic screening for bacteriocin activity using 1 769 human fecal isolates of *E. coli* and found that aside strains releasing phage particles, 300 (17%) secreted molecules lysing the indicator strain. Then, we further investigated 30 of these 283 strains and found that the supernatant of 20% of them induce a lambda prophage. Analysis of plasmid content associated with PCR screening for these 30 strains identified 77 bacteriocin encoding genes. Strikingly, only strains encoding toxins with endonuclease activity and the poorly characterized microcin Mcc1229 were prophage-inducers. The strain with the highest prophage-inducing activity displayed both an E-type endonuclease colicin and the Mcc1229 microcin, carried on two plasmids. By isolating each plasmid that were introduced into the MG1655 strain, we assessed the respective ability of these bacteriocins to induce various prophages in commensal lysogens. We observed similar inducing activity with a lambda prophage integrated into a second MG1655 strain, and we used this latter lysogen for co-culture experiments. As a result, the lysogen co-cultured with the endonuclease-producing strain showed increased prophage dynamics *in vitro,* with high lysogenization of the susceptible bacteriocin producer. However, no hints of a similar pattern were observed when introduced in germ-free mice.
Overall, the production of bacteriocins by *E. coli* intestinal isolates leads to frequent prophages induction from competitive bacteria. Yet, how this induction shapes gut phage dynamics remains to be determined. Our results focused on the E-type endonuclease colicin suggest that such effects may be spatially restricted within the gastrointestinal tract.

KEYWORDS: [prophage, induction, gut microbiota, bacteriocin]

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Latent glucosyltransferase functionality enables phage survival in hosts encoding multiple defense systems

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Bacteriophages encode diverse pathways to alter modify their genomic nucleobase composition. These modifications help phages to evade host antiphage defense systems such as restriction-modification (RM) and CRISPR-Cas systems. At the same time, modifications can also serve as the target for other host defense systems, which illustrates the complexity of the defense counter-defense landscape. Bacteriophage T4 encodes two glucosyltransferases (GTs), α-gt and β-gt, that post-replicatively add a glucose moiety to the hydroxy-methylated deoxycytosines (hmdC) on the phage DNA in an alpha- and beta-conformation, respectively. During a typical T4 infection, α-gt and β-gt contribute respectively to 63% and 37% glucosylation of all hydroxy-methylated cytosine residues. Here we demonstrate that, when present alone their glucosylation capacity increases to 80%, thanks to either their residual enzymatic activity or high transcription/translational efficiency, both of which are limited during infection by substrate availability. Encoding one of the two glucosyltransferases alone does not lower the ability of the phage to overcome *E. coli* RM systems, unless it lowers the glucosylation capacity below a 77-80% threshold. However, when encountering a host encoding the type I and type IV RM systems in addition to the DNA glycosylase Brig1, both phage glucosyltransferases are necessary for phage infection. These results demonstrate that encoding multiple glucosyltransferases with redundant functionalities provides an evolutionary advantage when simultaneously confronted with multiple antiphage defense systems.

KEYWORDS: Bacteriophage T4, glucosyltransferase; anti-defense mechanism; restriction-modification systems

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Targeting Shiga toxin-converting phage : from taxonomic analysis to protective defence systems

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Shiga toxin producing *Escherichia coli* (STEC) represent a group of enteric pathogens distinguished by their carriage of prophage-encoded Shiga toxins (Stx), which serve as the principal virulence factor and hallmark of STEC. Upon induction of the phage lytic cycle, Stx is released and exerts it ribotoxic effects through depurination of the highly conserved A_{2260} residue of the 60*S* subunit (1), thereby disrupting protein synthesis, and activating innate inflammatory responses.

Stx-encoding phages display considerable genomic and morphological diversity with genome sizes ranging between 30 kb to 122 kb (2,3) and spanning multiple taxonomic classifications (4). Despite this variability, they share a conserved genomic architecture and traditionally encode the Stx operon. In this study, we propose a revised classification framework for Stx-encoding phages that extends beyond the conventional reliance on Stx operon presence. By employing whole-proteome similarity clustering, we present the most comprehensive taxonomic analysis of Stx-encoding and Stx-like phages to date. This approach reveals critical insights into the phage host range and diversity across distinct *E. coli* phylogroups.

Additionally, our dataset enables the exploration of bacterial defence systems that may confer resistance to Stx-encoding phage infection. These systems are currently being experimentally evaluated. Ultimately, our findings aim to inform the development of targeted strategies to disrupt Stx phage transmission and mitigate the long-term health burden of Stx-mediated disease.

KEYWORDS: phylogenetics, STEC, taxonomy, defence systems, Shiga toxin

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If I am not selected for a talk, I would like to apply for a flash talk and poster.

Imaging of bacteriophage particle assembly *in vivo*: a path from the cytoplasmic membrane to warehouses in the compartmentalized bacterial cell

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** These authors contributed equally to this work*

Viruses are obligate intracellular parasites that require massive host resources to multiply efficiently. These manipulations lead to major cell remodelling including formation of viral-induced compartments for viral genome replication and/or assembly of viral particles. While viruses of eukaryotic cells can rely upon existing cellular structures, bacteriophages, the viruses that infect bacteria, have to multiply in an initial non-compartmentalized cytoplasm.

SPP1 is a strictly lytic phage of the Gram-positive bacterium *Bacillus subtilis*. The molecular mechanisms underlying the different steps of its viral cycle were well characterized *in vitro* but their integration in the crowded bacterial cytoplasm remains unclear. We showed that more than 300 copies of the viral genome are synthetized within the first 30 minutes after phage entry. These DNA molecules occupy an important space in the cytoplasm where procapsids proceed to SPP1 genome encapsidation before segregating in warehouses (Labarde et al, 2021). We then used cryo-FIB milling and cryo-electron microscopy (cryoET) to analyze the extensive spatial reorganization of SPP1-infected cells at single viral particle resolution in near-native conditions. The most prominent feature is the formation of a large viral DNA compartment from which ribosomes are excluded. This also allowed to image sequential steps of the viral particle assembly pathway that are confined to specific locations in the cell. In particular, we showed that procapsid formation initiates at the cellular membrane in a process that requires the presence of the portal protein. Open procapsid precursors remain associated to the membrane until procapsid completion. Procapsids then relocalize to the bacterial DNA compartment for DNA packaging. Finally, DNA-filled capsids leave this compartment for tail binding and clustering in warehouses until cell lysis. Altogether, we provide comprehensive mechanistic insights into the complete assembly pathway from membrane-associated precursors of procapsids to DNA-filled head-and-tail capsids and to their trajectory in the infected bacterium.

KEYWORDS: Viral-induced compartments, initiation of procapsid formation, portal protein, viral genome encapsidation, assembly pathway

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STRUCTURE AND ASSEMBLY

MOLECULAR SCALE

The auxiliary protein gp12 of bacteriophage SPP1, a model of prokaryotic collagens

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The genome of bacteriophage SPP1 encodes the auxiliary protein gp12, that binds at the center of the hexons formed by the major capsid protein (MCP) gp13. Gp12 is organized into three domains: an unstructured N-terminal region, a central flexible segment, and a C-terminal coiled-coil. The flexible region contains eight repeats of the Gly-X-Y motif, a hallmark of collagens (Zairi *et al*., 2014). Collagens are trimeric fibrillar proteins that play key roles in tissue stability in eukaryotes (Bella *et al.,* 1994). While extensively studied in eukaryotes, their structural and functional roles in prokaryotes remain largely unexplored.

We determined the crystal structure of gp12, that displays a tripartite architecture composed of an unstructured N-terminal region, a collagen triple helix, and a C-terminal coiled-coil domain. Furthermore, an icosahedral reconstruction of the mature SPP1 capsid revealed how gp12 anchors to the major capsid protein gp13. This interaction is mediated by salt bridges between basic residues in the N-terminal domain of gp12 and acidic residues of gp13.

Comparative genomics identified gp12-like proteins that share its modular organization, highlighting the unexpected prevalence of collagen-like domains in prokaryotic viruses. The structure of the gp12 collagen is stabilized by interchain interactions involving polar residues. A systematic bioinformatic survey of collagen sequences across the three domains of Life revealed that such polar residues are consistently overrepresented, supporting their general role in collagen stability. This finding provides a structural basis for collagen stability before its elaboration by hydroxylation of proline in eukaryotes.

Altogether, our results establish gp12 as a paradigm for phage-encoded collagens, expanding the repertoire of structural motifs in tailed phages and providing insights into the evolutionary conservation of collagen stabilization mechanisms.

KEYWORDS: Capsid structure, SPP1 bacteriophage, decoration protein, Collagen

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

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Bacteriophages are fascinating nanomachines that are highly specific and remarkably efficient in infecting their bacterial hosts.

While the cell enveloppe perforation mechanisms of the tail contraction 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 reconstituted 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.

We are using an integrative approach combining cryo-electron tomography (cryo-ET) and single particle cryo-EM to investigate the structure of T5 channel perforating the bacterial cell wall at different timepoints of infection.

KEYWORDS: Bacteriophage, Infection, Cryo-electron tomography, host-pathogen interaction

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Diversification of molecular pattern recognition in bacterial NLR-like proteins

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Antiviral STANDs (Avs) are bacterial anti-phage proteins related to the human immune pattern recognition receptors of the NLR family. Type 2 Avs proteins (Avs2) have been proposed to detect phage infection by recognizing a conserved and essential phage protein—the terminase large subunit—which has been suggested as the basis for their ability to defend against diverse phage families. We investigated an Avs2 protein from *Klebsiella pneumoniae* (KpAvs2) to determine the range of molecular signatures of infection it can recognize. To do so, we combined genetic approaches, AlphaFold structure prediction screening, and protein mass spectrometry during phage infection. We show that KpAvs2 recognizes multiple distinct phage proteins as signatures of infection. As previously suggested, KpAvs2 detects the terminase large subunit during infection by *Seuratvirus* phages. However, in *Dhillonvirus* phages, KpAvs2 targets a different protein of unknown function, which we name KpAvs2-stimulating protein 1 (Ksap1). KpAvs2 directly binds Ksap1 to become activated, and phages harboring mutations in Ksap1 can evade KpAvs2 defense despite encoding an intact terminase. Additionally, in *Justusliebigvirus* phages, KpAvs2 recognizes a third distinct protein of unknown function, named Ksap2. Our results demonstrate that the broad antiviral activity of KpAvs2 stems not from the recognition of a single conserved target, but from its ability to sense diverse, unrelated molecular signatures of infection. This highlights the evolutionary diversification of molecular pattern recognition mechanisms in bacterial innate immunity.

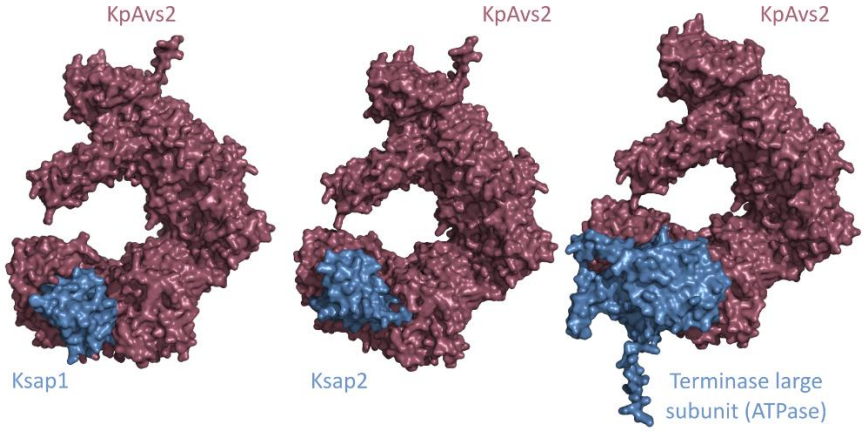

Fig. 1. AlphaFold prediction of KpAvs2 binding to three different phage targets

KEYWORDS: phage defence, pattern recognition, Avs

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Duplication-rich *Felixounavirus* genomes encode GlaF, a Gp2.5-like annealase defining a new family of phage recombinases

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Adaptation of the *Escherichia coli* LF82 virulent phage Felixounavirus P10 to new *E. coli* hosts in the gut involves duplication of sequences from tail fibre genes. Despite the lack of a recombinase gene in P10, short imperfect repeats flanking the duplicated segment suggested a recombinase-dependent mechanism.

To expand on this observation and search for a new recombinase candidate, a bioinformatics analysis of 5,226 complete phage genomes was conducted to identify duplications, recombinases and integrases in each genome. Duplications (>100 base pairs) were frequently found in phage genomes (23% of genomes), and particularly in tail genes, suggesting a possible role in phage evolution. Duplications also correlated with the presence of a recombinase, except notably in the genomes of Felixounavirus phages, which led us to investigate this family for a recombinase.

Using tools for protein remote homology detection and AlphaFold 3, we identified $Gp64_{P10}$ as a distant homologue of $Gp2.5_{T7}$, a single-stranded DNA binding and recombinase protein. Recombineering assays confirmed that $Gp64_{P10}$ acts as a recombinase, on ssDNA and dsDNA, the latter in cooperation with $Gp63_{P10}$ exonuclease. We conclude that $Gp64_{P10}$ is the first member of a new phage recombinase family conserved in Felixounavirus, GlaF, which, together with $Gp63_{P10}$, forms the first recombinase-exonuclease pair of the Gp2.5 superfamily.

Characterization of this new family of recombinases in phage genomes particularly prone to duplications reinforces the proposal that recombinases contribute to phage evolution by generating duplications.

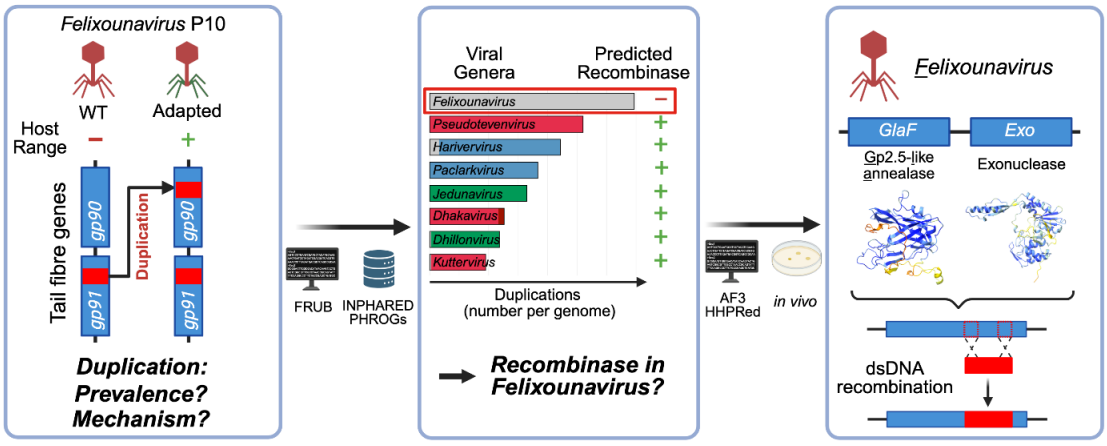

Fig. 1. Graphical abstract

KEYWORDS: Phage evolution; genetic duplication; tail fibre; recombination; Felixounavirus

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Empathi: Embedding-based Phage Protein Annotation Tool by Hierarchical Assignment

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Bacteriophages, viruses infecting bacteria, are estimated to outnumber their cellular hosts by 10-fold, acting as key players in all microbial ecosystems (Dion, M.B. in 2020; Yutin, N in 2018). Under evolutionary pressure by their host, they evolve rapidly and encode a large diversity of protein sequences (Labrie S.J. in 2010). Consequently, the majority of functions carried by phage proteins remain elusive (Pérez-Bucio, R in 2024). Current tools to comprehensively identify phage protein functions from their sequence either lack sensitivity (those relying on homology for instance) or specificity (assigning a single coarse grain function to a protein). Here, we introduce Empathi, a protein-embedding-based classifier that assigns functions in a hierarchical manner. New categories were specifically elaborated for phage protein functions and organized such that molecular-level functions are respected in each category, making them well suited for training machine learning classifiers based on protein embeddings. Empathi outperforms homology-based methods on a dataset of cultured phage genomes, tripling the number of annotated homologous groups. On the EnVhogDB database (Pérez-Bucio, R in 2024), the most recent and extensive database of metagenomically-sourced phage proteins, Empathi doubled the annotated fraction of protein families from 16% to 33%. Having a more global view of the repertoire of functions a phage possesses will assuredly help to understand them and their interactions with bacteria better. Finally, Empathi can be installed from https://huggingface.co/AlexandreBoulay/EmPATHi.

KEYWORDS: Protein functions; protein embeddings; machine learning; functional annotation.

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SOCIAL SCIENCES

Public Hospital-Based Phage Therapy in France: Rethinking Innovation Through a Public Health Lens

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[2]*Centre Emile Durkheim, Université de Bordeaux*
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This presentation examines the legal, regulatory, and policy challenges facing the development of phage therapy production in public hospitals in France, in the context of antimicrobial resistance (AMR), the urgent need for new antimicrobials, and the need to support alternative, public-interest innovation models.

Phage therapy—using bacteriophages to treat bacterial infections—has regained attention as a strategy against AMR. However, it remains largely incompatible with dominant Western pharmaceutical regulations, which favor standardized, commercially viable products. Phage therapies are highly personalized and difficult to patent, discouraging private investment and often making randomized controlled trials (RCTs) impractical. As a result, access remains limited outside of post-soviet countries like Georgia, where phage therapy has long been integrated into public health systems.

In response, some European countries have developed alternative models, localizing production and access within public hospitals and using more flexible regulatory pathways. This presentation explores the French case, comparing it with Belgium's magistral model, to examine how hospital-based innovation challenges pharmaceutical orthodoxy by disrupting the traditional separation between development and access. It also highlights the lack of political and financial support for sustaining public models of innovation.

Based on semi-structured interviews with researchers, policymakers, and regulators in France and Belgium (September 2023–December 2024), and an analysis of scientific and grey literature, this study shows how stakeholders creatively navigate legal and institutional barriers. It further situates the French phage therapy experience within broader debates about health sovereignty and essential medicine shortages in France and Europe, which have led to recent legal reforms enabling broader hospital pharmacy production.

Phage therapy, like other innovative treatments such as CAR-T therapies, signals the emerging role of hospitals as sites of pharmaceutical innovation, capable of developing personalized therapies in potentially more affordable ways.

KEYWORDS: Antimicrobial resistance; phage therapy; hospital innovation; pharmacy preparation; pharmaceutical regulation

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APPLICATIONS IN THERAPY AND BIOTECHNOLOGIES

Self-regenerating phage-loaded hydrogels: a synergistic prophylactic approach against staphylococcal skin infections

Emilie CENRAUD[1], Grégory FRANCIUS[1], Erwan ANDRE[1], Fabienne QUILES[1] and Xavier BELLANGER[1]

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Although phage therapy is promising, its clinical application is still limited by challenges in its implementation. Our project aims to propose a prophylactic approach focused on human and animal skin infections caused by *Staphylococcus aureus,* using hydrogels – safe and biocompatible materials that are already used as wound dressings. Our developed formulation of multilayered hydrogels combines long, charged polymers, such as hyaluronic acid, with poly(allylamine), which provides intrinsic antimicrobial properties. These hydrogels are designed to trap bacteriophages and release them in response to the presence of bacteria, thereby offering a synergistic mechanism of action in the form of a physical and chemical barrier complemented by targeted bacteriolysis (Figure 1).

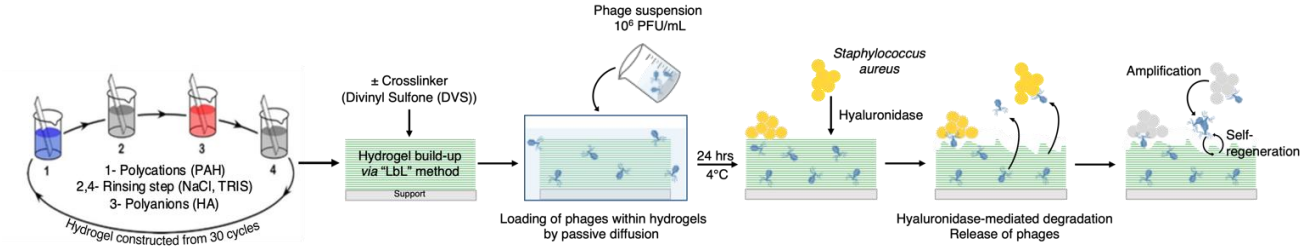

Fig. 1. Phage-loaded hydrogel system: bacteria-triggered phage release, and self-regeneration.

In the present work, we characterized 14 phages from different families, assessing their hydrophobicity, size, morphology and surface charge. Despite being phylogenetically close to each other, these properties varied substantially, influencing their interaction with the hydrogel matrix and their loading efficiency. Co-incubating individual phages with preformed hydrogels resulted in concentrations ranging from 10^6 to 10^8 PFU/mL of hydrogel, representing an up to 500-fold enrichment compared to the initial suspensions. This indicates that phage uptake follows an exponential dependence on phage size.

Chemical cross-linking was employed to modulate hydrogel stiffness; at optimal crosslinker concentration, the physical and chemical properties of hydrogels enhanced phage entrapment by up to 100-fold relative to non-crosslinked hydrogels.

Bactericidal activity was evaluated using two complementary methods: measuring bacterial adhesion to the hydrogel surface and determining the clearance of planktonic populations. First results indicate that crosslinked hydrogels without phages reduced bacterial adhesion by over 90% compared with glass controls (a ~2-log reduction), suggesting combined anti-adhesive and contact-killing effects. Incorporating phages further increased bacterial clearance, achieving a reduction of over 99% of planktonic cells, corresponding to 7-log depletion (release killing effect). Interestingly, phages released by lysed bacteria appeared to be re-sequestered within the hydrogel matrix, creating a self-regenerating reservoir that sustains high local concentrations of phages for an extended period of time. These results demonstrate that phage-loaded hydrogels combine intrinsic antimicrobial activity with self-regenerating phages, offering a robust system for the prophylaxis and therapy of *S. aureus* skin infections. Ongoing work aims to decipher triggered release, assess durability, and validate *ex vivo* and *in vivo* efficacy.

KEYWORDS: Hydrogel, Bacteriophages, *Staphylococcus aureus*, Hyaluronic acid, Phage therapy

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Activity of a cocktail of therapeutic phages in an *ex vivo* model of *Staphylococcus aureus* keratitis

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Background: This study aimed to evaluate two Silviavirus phages (V1SA19 and V1SA20), produced under GMP conditions, in an ex vivo keratitis model using *Staphylococcus aureus* clinical isolates. The study also aimed to assess the potential benefits of combining the Silviavirus phages with a Rosenblumvirus phage (SA_01).

Methods: The host range was determined using an efficiency of plating (EOP) assay and a liquid assay against multidrug-resistant (MDR) and methicillin-resistant (MRSA) clinical isolates of *Staphylococcus aureus*, collected from patients with keratitis. The phages were applied topically to a porcine cornea 20 hours after infection with clinical MRSA isolates, either alone or in combination, at 10-minute intervals for four hours. Vancomycin 2.5% was used as a comparator. The viable bacterial count was then determined six hours after treatment ceased.

Results: The EOP values were 12.5, 2.5 and 1.9 for SA_01, V1SA019 and V1SA020, respectively. All three phages achieved a lysis activity score (LAS) above the 75% threshold at a multiplicity of infection of 100. In the ex vivo keratitis model, combinations of SA_01 with either V1SA19 or V1SA20 resulted in a significant (>99.5%) reduction in bacterial load ($p < 0.001$). However, topical application of SA_01 alone ($p = 0.07$), V1SA019 alone ($p = 0.67$) or V1SA020 alone ($p = 0.09$) did not result in a significant reduction in bacterial load compared to untreated controls.

Conclusion: Overall, Rosenblumvirus and Silviavirus represent promising options for reducing viable bacterial counts in this ex vivo keratitis model. Combining one phage from each family produced a greater effect than using individual phage or vancomycin, achieving a reduction in the bacterial inoculum of over 99.5% compared with untreated controls. These results emphasise the topical therapeutic potential of phages in treating multidrug-resistant MRSA keratitis.

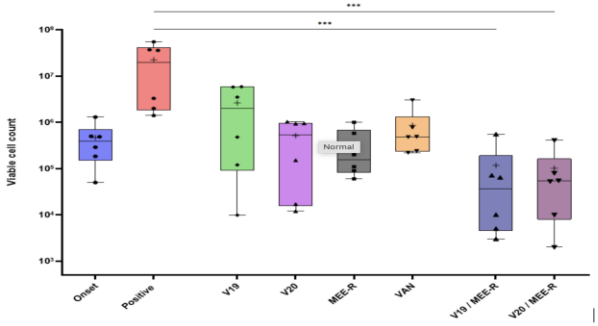

Figure – Box plot of viable bacterial counts in the ex vivo keratitis model after treatment with three phages (V19, V20, MEE-R), alone or in combination.
Bacterial load was measured after 20 hours of incubation, including 4 hours of treatment. A significant reduction in viable cell count was observed in the combination group (***$p < 0.01$, Dunn's test). VAN: Vancomycin 2.5%.

KEYWORDS: keratitis, phages, staphylococcus aureus, ex vivo
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PHAGEinLYON – THERAPhage : Isolation and Characterization of Bacteriophages Active Against *Pseudomonas aeruginosa* for Therapeutic Human Use

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Introduction. *Pseudomonas aeruginosa* (PA) is an opportunistic pathogen that is particularly associated with community-acquired and nosocomial respiratory infections. It is a major threat due to its multidrug resistance. Phage therapy is a promising alternative to antibiotic therapy. Using phage cocktails could help to limit resistance development, if the selected phages are sufficiently distinct and complementary. Within the THERAPhage project, our objective was to isolate anti-PA phages and assess their efficacy against a well-characterised collection of strains that are representative of the clinical and genetic diversity of PA, with the aim of evaluating their therapeutic potential.

Methods. Twenty-four phages were isolated from wastewater samples. Sequencing and analysis of phage genomes enabled the selection of 17 virulent phages. To assess their host range, 29 genetically characterized PA strains representative of the main infections-associated sequence-types, 12 strains isolated from bone and joint infections and 10 isolated from cystic fibrosis patients were tested. Phage susceptibility profiles were determined using the spot test and *Efficiency of Plating* (EOP) assays.

Results. Seventeen phages were selected after characterization (Figure 1). Twelve exhibited a *Myovirus* morphotype and belonged to five genera (5 *Pbunavirus*, 4 *Pakpunavirus*, 1 *Phikzvirus*, 1 *Litunavirus* and 1 *Nankokuvirus*), while five exhibited a *Podovirus* morphotype and were distributed across two genera (4 *Bruynoghevirus*, 1 *Phikmvvirus*). Four phages showed broad host ranges: one jumbo phage (*Phikzvirus* genus) showed the highest activity (EOP > 0,001 for 41% of strains), two *Bruynoghevirus* phages (EOP > 0,001 for 35–39%), and one *Pakpunavirus* phage (EOP > 0,001 for 27%). Together, these four phages covered 74% of the tested strain panel.

Conclusion and Discussion. We present the characterization of a phage collection with complementary host ranges against a diverse set of PA isolates. Current work focuses on (i) phage training to expand the host range of *Bruynoghevirus* and *Pakpunavirus* phages and (ii) the development of a GMP production process using a production strain free of prophages and major virulence factors, in accordance with ANSM requirements for human therapeutic use.

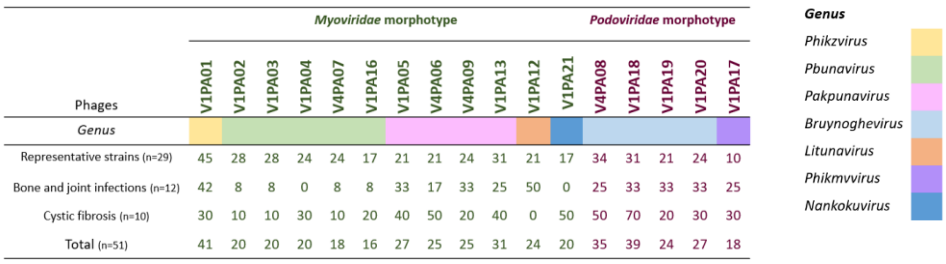

Figure 1. Activity spectrum of the seventeen virulent phages against a collection of 51 clinical strains of PA. For each phage, the numbers indicate the % of strains with an EOP ≥ 0,001.

KEYWORDS: *Pseudomonas aeruginosa*, antibiotic resistance, bacteriophages, characterization

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Phage-derived endolysins enable targeted editing of the gut microbiota and uncover niche overlap between oral and butyrate-producing taxa

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The small intestine plays a vital role in digestion, nutrient absorption, immune regulation and it is home to a unique microbial ecosystem. We previously described a distinct form of dysbiosis termed small intestinal oral bacterial overgrowth (SIOBO), characterized by ectopic colonization of oral taxa such as *Streptococcus salivarius*. SIOBO has been associated with impaired nutrient uptake, gut barrier dysfunction, and stunted child growth (Yersin, S. and Vonaesch, P., 2024). Given the global burden of undernutrition, we sought to develop precision tools to target key detrimental bacteria contributing to this condition. Bacteriophages and their enzymes have recently seen a resurgence as tools to specifically target pathogenic and commensal bacteria. From 40 upper gastrointestinal isolates obtained from stunted children in Sub-Saharan Africa, we computationally identified two high-quality prophages, which we successfully induced and visualized via electron microscopy. Genome sequencing confirmed them as novel *Siphoviridae* phages (~43 kb). The induced phages did not produce clear lysis plaques on any of the target clinical isolates of *S. salivarius* tested. As an alternative, we thus established a streamlined protein expression pipeline to produce their lysins, key enzymes for bacterial lysis by the phages. Using Gibson Assembly, we produced a lysin with potent and specific activity against 37 out of 40 *S. salivarius* isolates, sparing closely related species such as *S. mitis*, *S. parasanguinis*, and *S. thermophilus*. Using stool-derived *in vitro* communities, lysin treatment specifically reduced *S. salivarius* abundance and indirectly promoted *Coprococcus comes*, a beneficial butyrate producer linked to healthier child growth. This inverse relationship led us to hypothesize that *S. salivarius* negatively interacts with *C. comes* and possibly with other beneficial butyrate producers. We confirmed a metabolic niche overlap using spent medium interaction assays involving 32 additional butyrate-producing strains. *In vivo*, two-doses of lysin treatment in colonized mice reduced *S. salivarius* ~2-4 fold. Combined with complementary nutritional interventions, the use of phage-derived lysins against key members of SIOBO may offer a precision medicine strategy to remodel a dysbiotic microbiota and improve growth outcomes in children. This approach could also provide a promising framework to precisely target a broader range of intestinal pathogenic bacteria.

KEYWORDS: Phage endolysins; microbiota engineering; ectopic colonization; bacterial interactions; niche overlap.

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FLASH TALKS & POSTERS

2025 PHAGES IN NANCY

ECOLOGY AND EVOLUTION

POPULATION SCALE

Viruses in your cider? Uncovering new phages in fermentation.

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Bacteriophages (or phages) are viruses infecting specifically bacteria. Despite their relevance, phageomes (phages communities) remain poorly understood, particularly regarding their roles within microbial ecosystems. In fermented beverages, phageomes are thought to significantly influence microbial population structures and dynamics, especially in a temperature-dependent manner [1]. The current study aims to deepen our understanding of phage diversity in fermented beverages, using cider as a case study.

Between October and December 2024, cider samples were collected from 12 producers in Normandy region (France), resulting in 24 samples taken at early fermentation stages. Microbial isolation and enumeration were carried out using 9 different culture media, and isolates were identified by MALDI-TOF. To improve phage detection, the pH of each cider was adjusted to 7. Samples were centrifuged and filtered (0.45 µm), and spot tests were performed using the filtrates against the isolated bacterial strains from the same ciders. The phages obtained were analyzed for their host range, morphology by transmission electron microscopy, and genome content.

The screening of 366 bacterial isolates resulted in the isolation of 17 phages from five cider samples. Sixteen phages infected acetic acid bacteria of the genera *Gluconobacter* and *Acetobacter*, while one phage targeted *Rahnella aquatilis*. Most were myophages, with contractile tails, except for the *Rahnella* phage, which was a podophage with a short non-contractile tail. Notably, five phages infected *Gluconobacter cerinus*, a species previously associated with only one known phage GC1 isolated from wine [2], while the remaining phages targeted isolates from the species *G. thailandicus/frateurii*.

These newly isolated cider phages appear novel and are currently undergoing further genomic characterization. Next, a model microbial community will be developed to study phage–bacteria interactions during fermentation process.

KEYWORDS: Bacteriophages, phageomes, cider, acetic acid bacteria, fermentation

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Role of prophages in antiviral resistance in *Pseudomonas aeruginosa*

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The prophages which lie dormant in the genomes of bacteria can associate with their hosts multigenerationally, and thus are likely to experience multiple infection events from other phages. However, co-infection dynamics of multiple phages in one host is understudied. The co-mingled fates of prophage and host means there is an evolutionary interest for the prophage to protect the host during co-infection. Bacteria can protect themselves by either mutating genes essential for phage life-cycles, or expressing genes designed to destroy/halt phages. However, curiously these defence genes are often encoded on mobile genetic elements (MGEs), which suggests inter-MGE competition as a key driver of the evolution of defence systems. Prophages in particular constitute a rich source of defence systems, thus a potential vector for the dissemination of defence genes in populations. Using a collection of prophages and virulent phage (which cannot integrate into the host genome) in *Pseudomonas aeruginosa* we have identified prophages that provide resistance against virulent phage lysis to their host. Some prophages provide broad resistance against many virulent phages, while others provide resistance to a few virulent phages in an environmentally dependent manner. Finally, some virulent phages are sensitive to a broad range of prophage strains. Interestingly, these virulent phages also display temperature sensitivity, suggesting more global aspects of virulent phage physiology that leave them vulnerable to prophage-mediated resistance. We are using representative prophage-virulent phage pairs to investigate how prophage-encoded defence genes impact the selection and spread of co-infecting phages in a bacterial population? Furthermore, how the virulent phage can co-evolve and evade these prophage-mediated resistance types? Prophage and co-infection are abundant in natural settings, thus understanding the evolutionary interplay between MGEs is important for therapeutic and agricultural uses of phage.

KEYWORDS: co-infection, phage competition, defence systems

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Abundance and diversity of phage populations in different cheese varieties

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Bacteriophages are ubiquitous and are considered a key element in the assembly of microbial communities and, more broadly, in the functioning of ecosystems. Fermented foods, such as cheese, contain dense and diverse microbial communities and are therefore an ideal biotope for the development of these viruses. Initial research conducted on a specific variety of washed-rind cheese, employing novel viral metagenomic methodologies, has unveiled that, in addition to the well-studied lactic acid bacteria (LAB) phages, cheeses harbour a diverse array of phages that infect ripening bacteria (Dugat-Bony et al., 2020; Paillet et al., 2022).

The objective of this study is to obtain a broader perspective on the abundance and diversity of phages within the cheese habitat. In order to achieve this objective, a comprehensive sample set comprising 70 cheeses, belonging to distinct varieties, was collected and analysed.

Viral particle counts were performed using epifluorescence microscopy, and the viral load was estimated to range from 9.3×10^6 to 7.4×10^9 VLP/g of cheese surface. A significant heterogeneity was observed depending on the type of cheese studied, indicating a possible impact of cheese-making technology on the abundance and multiplication of phages in cheese.

Concurrently, viral particle extracts were prepared from cheese samples and utilised for the isolation of phages. At this stage of the project, 37 isolates have been obtained, infecting bacteria commonly found on the surface of cheese, such as *Corynebacterium casei*, *Staphylococcus equorum*, *Brevibacterium aurantiacum* and *Glutamicibacter arilaitensis*. The morphotype of the phage isolates was determined by means of transmission electron microscopy. Completion of this work will be achieved by analyses of their genome, host spectrum and infectivity.

The creation of this collection of phages infecting ripening bacteria paves the way for a better understanding of these viruses. In the next stage of the project, they will be used to assess their impact on the assembly of bacterial communities during ripening and on cheese quality.

KEYWORDS: Bacteriophage, cheese, ripening bacteria

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Phage-Bacteria Dynamics within the Mucosal Environment

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The mucus layer is an essential part of the gut barrier for protection against pathogens and maintaining of the intestinal homeostasis. This layer predominantly consists of mucin (MUC2) proteins which form a dense, stratified physical barrier that separates the epithelium from the gut lumen. It is known that mucolytic bacteria are essential to maintaining the homeostasis of this layer and that bacteriophages contribute to global bacterial diversity. This project aims to characterize the interactions between bacteriophages and bacteria in the mucus layer.

The biogeography of bacteriophage infections, their replicative capacity and their dynamics of coexistence with their host bacteria is studied in a novel in vitro model using mucin beads in culture media to mimic lumen-mucus interface. Our preliminary results show that *E. coli* colonizes inside the mucus beads (10^7 CFU/g of mucin) for at least 4 days and that *E. coli* phages (M13 and T4) reside in the beads in absence of bacteria. Plate enumeration and confocal microscopy showed that phages actively replicate (to up to 10^{10} PFU/g of mucin) and reach deeper mucus layers when bacterial hosts co-colonise the beads, suggesting a "carrier" state allowing phages to co-localise with their hosts.

Mucolytic bacteria *A. muciniphila* and *B. thetaiotaomicron* also colonise the mucin beads in anaerobic conditions (10^7 CFU/g of mucin and 10^8 CFU/g of mucin, respectively) at similar levels to those seen in the media. Together, these preliminary results prove that the model is suitable for studying the ecological dynamics occurring at the interface between the intestinal lumen and its outer mucus layer.

Current work is focusing on comparing the infectious capacity of phages over time in the liquid versus the mucosal compartment to enlighten specific dynamics of phage/bacteria coexistence. Characterizing phage-bacteria interactions in the mucus layer will offer a key to understanding these dynamics in a context of barrier defect and dysbiosis such as IBDs.

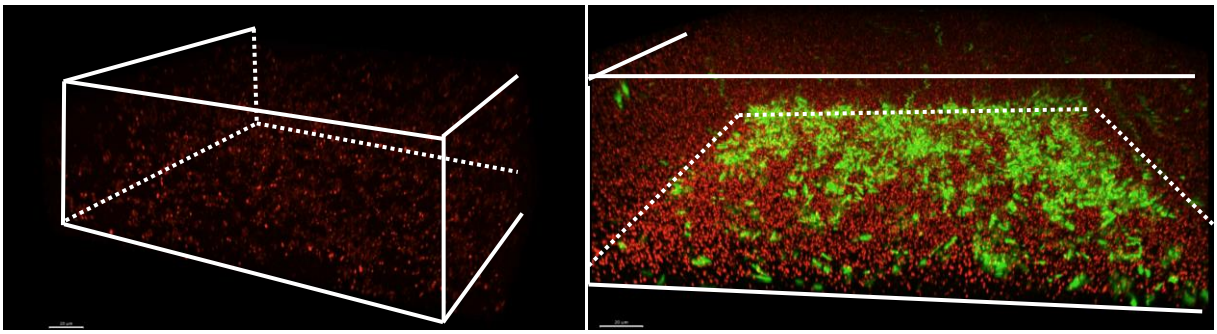

Fig1: Images from confocal microscopy showing bacteriophages M13 (in red) and *E. coli* MG1655 (in green) inside a mucin II bead (contours are marked by white lines).

KEYWORDS: Bacteriophage-Bacteria dynamic; Mucosal environment; Lumen-Mucus interface; Bacteriophage Adaptation

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OCCIPHAGE: Exploring phage diversity for a sustainable biocontrol of *Xanthomonas* crop pathogens

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Bacteriophages, viruses that specifically infect bacteria, are promising biocontrol agents against phytopathogens such as *Xanthomonas spp.*, which threaten crops of high economic importance. Current control relies on copper-based compounds, whose long-term accumulation raises environmental concerns, highlighting the need for sustainable alternatives.

The Occiphage project aimed to explore the diversity of phages in the Occitanie region and assess their potential against two key bacterial pathogens: *Xanthomonas phaseoli* pv. *manihotis* (Xpm), the agent of cassava bacterial blight, and *X. campestris* pv. *campestris* (Xcc), responsible for black rot in Brassicaceae. Environmental samples from wastewater and irrigation water were screened for lytic phages. In total, 67 virulent phages active against Xpm and 9 against Xcc were isolated, with host range evaluation identifying candidates effective against most pathogen strains. Genomic sequencing of representative isolates revealed their diversity and provided a foundation for understanding phage–host interactions.

This work establishes a framework for phage-based biocontrol and highlights the potential of bacteriophages as sustainable alternatives to chemicals in managing *Xanthomonas* diseases.

KEYWORDS: Agriculture, Biocontrol, Phytopathogenic bacteria

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1st year of EPIPHAGES-OI project: Extended EPIdemiological surveillance of the Ralstonia solanacearum species complex and their bacterioPHAGES as natural antagonists – presents in the Indian Ocean

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The *Ralstonia solanacearum* species complex (RSSC) is a devastating Gram-negative plant pathogen causing bacterial wilt in more than 250 plant species, including economically important Solanaceae such as potato, tomato, eggplant, and pepper. Listed as a quarantine organism by EPPO (A2 List, 2023), RSSC exhibits remarkable genetic and phenotypic diversity, with four phylotypes and over 70 described sequevars, making disease management highly challenging.

Given the absence of efficient and authorized control methods, the development of environmentally friendly alternatives is a major priority. The South-West Indian Ocean (SWIO) islands are recognized as a hotspot of genetic diversity for both the RSSC and their associated bacteriophages, offering a unique opportunity to explore innovative strategies [Yahiaoui et al. 2017, Rasoamanana et al. 2023, Trotereau et al. 2021]. In this context, the EPIPHAGES-OI project investigates phage-based biocontrol as an eco-friendly strategy, since bacteriophages are natural viral antagonists of bacteria, abundant and ubiquitous in ecosystems, and already considered promising tools for the management of plant pathogenic bacteria (Clavijo-Coppens et al. 2022). Launched at the end of 2024 for a two-year period, the project is supported by the French Biodiversity Agency under the ECOPHYTO program, which seeks to reduce pesticide use in French agriculture. Its main objective is to strengthen the epidemiological surveillance of RSSC by integrating naturally occurring phages under diverse agricultural conditions in Réunion and Mayotte.

During the first year, 30 sites were surveyed on Réunion Island across tomato, eggplant, pepper, and potato cultivation areas, covering heterogeneous agronomic and climatic conditions. This led to the collection of 97 RSSC isolates and 33 RSSC-associated phages. PCR screening on isolated bacteria confirmed the coexistence of phylotypes I and II, with a predominance of phylotype I. *In vitro* interaction assays revealed that 62% of the isolated phages are generalists, infecting more than 90% of isolates tested, while two phages showed narrower host ranges (infecting respectively 16% and 67% isolates). Interestingly, phage sensitivity varied even among isolates collected from the same site.

Ongoing genome sequencing of both bacteria and phages aims to (i) establish epidemiological links between their genetic diversities and (ii) generate a spatial and temporal interaction map. These outcomes will contribute to developing decision-support tools for the design and deployment of phage-based biocontrol products. Altogether, the phages reported here represent promising candidates for the sustainable management of bacterial wilt caused by RSSC. These results will be made available to scientists, producers, and agricultural decision-makers through an online application currently under development (link: https://fcc-cirad.quarto.pub/epiphage-oi/).

EPIPHAGES-OI Project

KEYWORDS: *Ralstonia solanacearum*, Phage-Based Biocontrol, Phage Ecology

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PHAGE-HOST INTERACTION

CELLULAR SCALE

Characterization of CtMAD Domain in MuF Phage Proteins: A Potential Factor in Phage-Host Interactions

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Polymorphic toxins are characterized by a multi-domain organization and are often involved in interbacterial competition. MuF polymorphic toxins are specifically encoded by temperate bacteriophages and their prophages, and are defined by a conserved N-terminal MuF domain and a variable C-terminal domain. MuF proteins remain poorly characterized, although they are believed to exist in 2–3 copies within the virion head and to bind viral DNA. The major part of MuF proteins lack the C-terminal domain and are referred to as the short form. However, approximately one-third of MuF proteins do possess a C-terminal domain, which can be either toxic or of unknown function. Notably, among the C-terminal regions with unknown function, one-third correspond to the CtMAD domain for C-terminal MuF Associated Domain. The conservation of this domain among various prophage genomes indicates it may play an important role, possibly in mediating phage-host dynamics or bacterial competition. However, the exact biological role of the CtMAD domain remains undefined. In this study, we aim to determine the activity and role of this unknown domain, identifying its targets and elucidating its potential function in phage biology.

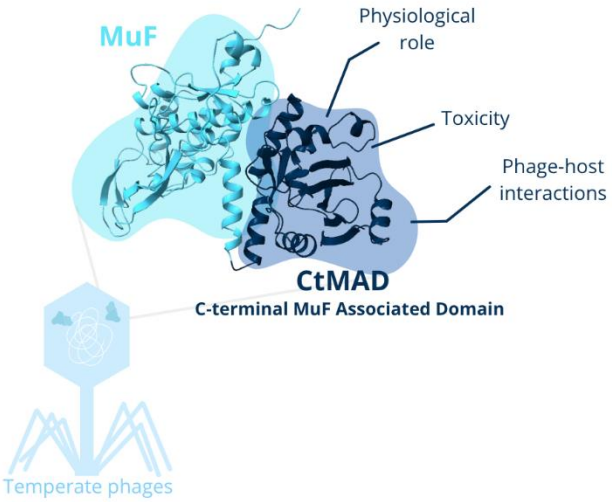

Fig.1. Structural representation of MuF protein carrying CtMAD domain and associated functional questions.

Keywords: MuF proteins, CtMAD domain, Temperate bacteriophages, Phage-host interactions, Prophage-encoded effectors.

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Regulation of anti-phage systems: a single-cell approach in *E. coli* F

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Through coevolution, bacteria have developed anti-phage defense systems (APDS), while bacteriophages have acquired systems to circumvent cellular defenses. Numerous bacterial defense systems have been predicted, and the molecular mechanisms of some have been studied (Georjon and Bernheim, 2023). However, their regulation remains poorly understood. Our objective is to study the expression conditions of the genes encoding several of these defense systems using single-cell approaches: are they expressed constitutively, or are they activated or repressed under certain environmental conditions or upon phage infection? Is there co-regulation of several systems suggesting functional synergy?

Our study model is, on the one hand, the *Escherichia coli* F strain, which has four APDS identified to date: type I restriction-modification (RM), Bacteriophage Exclusion (BREX) type I, AbiH and Zorya type II, the latter being defective. On the other hand, *Tequintavirus* T5, which is insensitive to APDS of strain F, *Felixounavirus* Yueh, which is sensitive to BREX, and *Tunavirus* Ghanima, which is sensitive to RM (the latter two phages were isolated by the team).

We have already constructed biosensors in which the structural genes of one or more defense systems have been replaced by a fluorophore gene, allowing us to monitor the activity of the promoters of these systems at their actual genetic loci. In a second step, we plan to build biosensors in which the structural proteins of APDS will be fused to fluorophores in order to study the production, degradation, and localization of APDS proteins. We have infected the biosensors with different phages (WT, capsid labeled with a fluorophore, or expressing a fluorescent protein in the early stages of infection). In this poster, we present the results obtained with different biosensor/phage combinations on the expression of APDS from strain F.

From the bacteriophage's point of view, a transcriptome analysis (RNA-seq) carried out by the team revealed that at least one pre-early gene of T5 prevent the expression of the structural genes of the RM and BREX systems. To study this regulation, we combine microscopy with Single Molecule Inexpensive Fluorescence In Situ Hybridization technique (tracking mRNAs during infection and host chromosome degradation). In order to identify which early gene(s) of T5 is responsible for this regulation, we are developing a CRISPR-interfering (CRISPRi) approach that uses dCas9 and a specific guide RNA in strain F to block the transcription of candidate phage genes. To validate this approach, we present the effect of a specific guide RNA that represses the expression of the *A2* gene that encode an essential pre-early DNA binding protein in T5 WT: the absence of A2 production results in phage DNA injection blocked at the First Step Transfer (*i.e.*, no virion production and no cell lysis). This strategy, combined with smiFISH, will enable the identification of T5 early genes involved in the repression of BREX and RM genes in the F strain.

KEYWORDS: Phage/ bacteria coevolution, bacterial defense systems, regulation, transcriptomics, single cell

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Distinct endocytosis pathways mediate hepatic uptake of bacteriophages

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The gut–liver axis represents a critical communication pathway that is disrupted in inflammatory bowel diseases (IBD), where compromised intestinal barrier integrity facilitates gut microbiota translocation to the liver, through the portal vein. Among these microbes, bacteriophages (phages) constitute the most abundant viral population. Our team has shown that phages can translocate across the intestinal barrier (Douadi *et al.*, 2024). However, their impact on liver physiology remains unknown. This study investigates the mechanisms of phage endocytosis in hepatic epithelial cells under physiological and inflammatory conditions. Hepatocyte (HepG2) and cholangiocyte (H69) cell lines were exposed to three morphologically distinct phages (T4, M13, ϕX174). Phage endocytosis was characterised *via* plaque assays, confocal microscopy, and pharmacological inhibition of endocytic pathways. Results show differential phage internalization, with greater uptake in cholangiocytes compared to hepatocytes, and progressive accumulation over time. The process appears to be independent of clathrin-mediated endocytosis but dependent on dynamin activity, varying depending on the phage type. Under lipopolysaccharide (LPS)-induced inflammation, phage uptake was enhanced in hepatocytes, in a phage-dependant manner. These findings reveal that phage uptake in hepatic epithelial cells is both phage- and cell type–dependent, underscoring distinct endocytic pathways that govern phage–liver interactions.

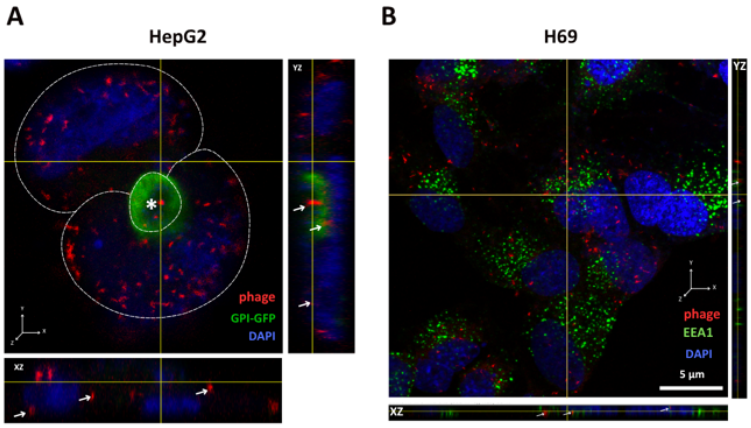

Fig. 1. Internalization of the M13 bacteriophage in physiological conditions after 4h. M13 phage (red) internalized in the hepatocytes (HepG2)(* = bile canaliculus) (**A**) and cholangiocytes (H69) (**B**).

KEYWORDS: gut-liver axis, endocytosis, bacteriophages, hepatocytes, cholangiocytes

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Isolation and characterization of a phage collection against *Pseudomonas aeruginosa* of veterinary origin.

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Pseudomonas aeruginosa is a ubiquitous bacterium but also an important opportunistic and nosocomial pathogen in humans, involved in healthcare-associated pneumonia, cystic fibrosis or burn wound infections. In companion animals, particularly in dogs, *P. aeruginosa* is commonly responsible for a variety of difficult-to-treat infections like pyodermatitis or otitis. Although *P. aeruginosa* does not often cause life-threatening diseases in animals, treating this pathogen is difficult first due to its capacity to form biofilms and to resist to numerous antibiotics through intrinsic and acquired mechanisms, and second due to the limited number of molecules available in the veterinary therapeutic arsenal. Therefore, using phages combined with antibiotics might be an interesting alternative to treat otitis in dogs.

Between 2019 and 2023, 256 *P. aeruginosa* isolates responsible from dog otitis were collected through the Resapath network and whole-genome sequenced (Illumina). Results showed a high genetic diversity, with 162 different STs identified, among which only eight were shared by more than five isolates. Very few acquired resistance genes were identified, but phenotypic resistance against carbapenems (meropenem and/or imipenem, n=22) and amikacin (n=20) suggested mutations in efflux pumps. From this collection, we selected 32 *P. aeruginosa* isolates belonging to the most frequent STs and/or presenting resistance to carbapenems. Phages active against these 32 isolates were isolated from water samples collected in the wastewater treatment plant (WWTP) of Reims.

In total, 28 lytic phages were isolated, purified and their titer was determined. The host range of all 28 phages was determined against the 32 selected *P. aeruginosa* isolates. Subsequently, the host range of the 15 most efficient phages was assessed against the whole (n=256) collection of isolates from dog otitis. In parallel, the DNA of the 28 lytic phages was extracted using the phenol-chloroform method and sequenced (Illumina). After quality controls, reads were assembled using Unicylcer (BVBRC site) and annotated using Pharokka v1.7.5. Molecular investigations revealed that they all presented dsDNA genomes, whose size ranged between 39°621pb and 66°443pb. A blastn comparison of the sequenced phage genomes with the NCBI database showed that they were related to the Caudovirecetes class, and associated to four different families and six different genus. These data were corrobated by a kmer-based Mash analysis.

Based on the phenotypic and genomic results, the 10 phages (which belonged to three different genus: bruynoghevirus, phikmvvirus, pbunavirus) presenting the largest host range and non-identical genomes were further characterized for their infection characteristics (one-step growth curves, adsorption rate constant, thermal and pH stability assay). Phages showed similar results for thermal (between 4°C and 45°C) and pH stability (between pH 4 and 10) assays, except for one phage which was still viable at pH 2 or 12 and at 60°C. On the contrary, they presented distinct growth characteristics. Further characterizations (including MinION sequencing) are ongoing.

This study proved that lytic phages with large host range against *P. aeruginosa* of veterinary origin can be isolated from WWTP. These newly isolated phages are promising tools in a sector where the therapeutic arsenal is limited, and they might be used in future clinical trials. It would also be of interest to determine if these phages are also effective on *P. aeruginosa* of human origin, thus expanding the Global Health approach to infection control.

Ag85A-dependent lipid remodeling impairs therapeutic mycobacteriophage adhesion

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Patients infected with mycobacteria, particularly *Mycobacterium abscessus*, frequently experience antibiotic treatment failure. Phage therapy, used under compassionate care, offers a promising alternative and has already demonstrated clinical efficacy (Dedrick, RM, 2023). However, phage-bacteria interactions and their co-evolution remain critical factors that can compromise therapeutic success. Trehalose polyphleate (TPP), a complex lipid of the mycomembrane, plays a key role in the adhesion of two therapeutic mycobacteriophages, BPs and Muddy (Wetzel, KS, 2023). Loss of TPP synthesis confers bacterial resistance, which can be circumvented by phage adaptation of minor tail proteins.

In this study, we found that deletion of the *Ag85A* gene, encoding an enzyme involved in trehalose dimycolate (TDM) biosynthesis from trehalose monomycolate (TMM), impaired BPs and Muddy activity in both solid and liquid media. Adhesion assays and flow cytometry demonstrated a defect at the binding stage, supported by lipid profiling that revealed alterations in the first barrier encountered by phages. Notably, accumulation of TMM and TDM was observed in the deletion mutant. Together, these findings suggest that TPPs, essential for BPs and Muddy infection, may be masked by excess trehalose-bound lipids, subsequently reducing phage access and thereby preventing effective bacterial recognition and adhesion.

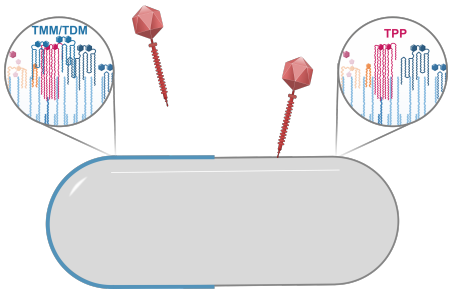

Fig. 1. Overproduction of TMM and TDM masks TPP, lipids required for mycobacteriophage adhesion

KEYWORDS: Bacterial resistance, Mycobacteria, Antigen85A, Mycomembrane lipids, Adhesion

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STRUCTURE AND ASSEMBLY

MOLECULAR SCALE

Termini and packaging identification using PhageTermVirome software with Nanopore data

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PhageTermVirome (PTV) is the update of PhageTerm, for the analysis of multiple phages (by R. Garneau, J in 2021). This software helps for genome analysis by allowing the characterisation of phage termini and packaging mechanism from sequencing. Using short-read sequencing data, it detects coverage biases and uses a statistical method to give robust results. It is based on random fragmentation during library preparation wich allows the coverage bias detection. PhageTermVirome can be used for large-scale studies as it can work on large virome datasets. Parallelization options have been added so it can handle over 5000 different phages in a simultaneous way.

In this project, we investigated if PhageTermVirome is able to handle long-read data from Nanopore sequencing technology. We would like to share interesting results about the parameters we tested, which allow PTV to work with Nanopre long-reads. We have some good practices to share about library preparation and bioinformatics preliminary steps to get the best performance from PTV.

We confirmed that using ligation kit, random DNA fragmentation is optional and can be skipped. DNA input can also be decreased up to 0,04ng, allowing the sequencing and PTV analysis on many viromes with low DNA yields (by Sbaghdi, T in 2025). We tested Autocycler, Flye and Spades to get different assemblies that PTV can use for mapping. Among the assemblers tested, our results suggest that Spades is only approach that allows PTV to find termini and packaging mechanism for all of the 5 tested phages. Finally, we developed a new process for prefiltering reads from large virome data to get PTV analysis faster.

KEYWORDS: software, long-reads, termini, packaging, virome

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Interaction between the *Neisseria meningitidis* type IV pili machinery and the MDA bacteriophage extrusion system

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Neisseria meningitidis (*Nm*) is a human commensal bacterium responsible for meningitis and septicaemia. The first step of infection is the crossing of the nasopharyngeal barrier followed by proliferation into the bloodstream. The two main virulence factors are the polysaccharide capsule which protects *Nm* from complement attack and the type IV pili (T4P). T4P are essential for adhesion, competence, aggregation, and motility. Although these virulence factors are necessary, they alone do not explain the hyperinvasiveness of *Nm*. We have already shown that a filamentous bacteriophage, MDAΦ (Meningococcal Disease Associated), is associated with invasiveness and disease in adolescents (Bille et al. 2005). It is integrated into the *Nm* genome and produces viral particles without lysing *Nm*. It has a primordial role in the colonization of the epithelium by forming a network around bacteria, like a matrix (Bille et al. 2017).

The aim of this study is to characterize the MDAΦ secretion machinery and to establish its bacterial and viral parts. Firstly, we characterised the T4P machinery genes involved in secretion. PilQ, PilW and TsaP are the only proteins among those tested as being involved in MDAΦ secretion. On the phage side, we had previously shown that ORF8 is a phagic ATPase responsible for the MDA assembly. Using Alphafold, we predicted the conformation of ORF8 like a multi-protein complex and established the possible interaction between ORF8 and PilQ N0 domain. We confirmed this interaction by double hybrid assay. We suggest that a new protein called ORF11 is encoded within the ORF8 frame and allows interaction with the bacterial secretin pilQ. We are currently working on highlighting it.

Finally, we hypothesize that the formation of the phage secretion machinery hijacks the T4P machinery. The ORF8 overexpression in *Nm* devoid of MDAΦ leads to inhibition of piliation.

This study aims to develop an in-depth molecular understanding of MDAΦ secretion, its use of the T4P machinery and the impact of the ORF8/PilQ complex on T4P secretion.

KEYWORDS:

Filamentous bacteriophage, Virulence, Neisseria meningitidis, type IV pili, secretin

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SOCIAL SCIENCES

From Lab to Field: What's Holding Back Phage-Based Biocontrol?

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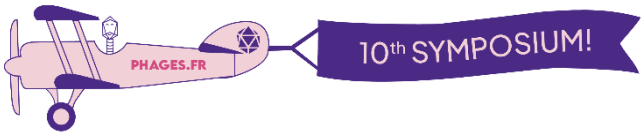
Our proposition aims to identify the main obstacles to innovation in agricultural phage therapy within the European context, and explore the institutional and organizational levers that could help overcome them. Despite the real potential of phages to control certain phytopathogens, turning them into an effective biocontrol solution is currently hampered by misaligned regulatory frameworks and established practices that create bottlenecks (see Malausa T et al. in 2024). Reducing the use of chemical pesticides and promoting environmentally friendly biocontrol strategies requires more than laboratory breakthroughs or experimental results. A full understanding of the socio-technical challenges at stake demands attention to the entire chain of actions involved in turning phages into an agricultural innovation.

Our analysis draws on an interdisciplinary research project - bringing together microbiologists and sociologists - aimed at developing a practical biocontrol solution while also considering how a community of (future) users might take shape. Beyond the well-studied scientific and regulatory dimensions, our approach highlights the constraints and tensions that shape each stage of the process, from phage discovery to commercialization. In our poster we will expose three key contributions to current debates on phage therapy in agriculture (according, first, to the need for infrastructures that make phage efficacy visible, measurable and assessable; second, to the economic viability of phages; and third, to open innovation - engaging diverse actors across the entire process). While focused on phages, our framework applies more broadly to microbial innovation for sustainable agriculture.



KEYWORDS: bacteriophages, biocontrol, innovation, obstacles, levers

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POSTERS

2025 PHAGES IN NANCY

ECOLOGY AND EVOLUTION

POPULATION SCALE

Frequent and rapid turnover of defence systems in P2-like prophages and their P4-like satellites

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Microbial ecosystems are shaped by complex interactions between bacteria, bacteriophages and phage satellites. The latter are non-autonomous elements that mobilise by hijacking viral particles of phages (Ares-Arroyo, M 2024). Although phages are bacterial parasites, and satellites exploit phages, the interests of all three elements can sometimes be aligned. One key example is the presence of antiviral defences in P2 prophages and P4 satellites, which protect their common host bacteria from predation by other phages, thus contributing to the survival of the trio (Rousset, F 2022). The turnover of antiviral immunity in bacteria relies on the presence of these genes in mobile elements (Aysha Hussain, F 2021), but less is known about the turnover and evolutionary dynamics of bacterial defences within the mobile elements themselves. Here, we harness the recent discovery of thousands of P4-like satellites (Moura de Sousa, J 2023) and P2-like prophages, to glimpse at how bacterial immune systems shape the evolution and interactions of these mobile elements between themselves, and with their bacterial hosts. We confirmed that defence systems are prevalent in almost all P4 and P2 genomes, and are a substantial fraction of the pangenomes of these mobile elements. We observed that defence genes diversify very rapidly in P4 and P2 genomes, likely through direct swaps with different defence systems. Although we show that immunity genes can be exchanged with different mobile elements (e.g., plasmids), these exchanges rarely occur between P4 and P2. Our focus on defence systems in the context of the tripartite bacteria-satellite-phage interactions sheds light on the ecological, evolutionary and genomic dynamics of the trio, and highlights the role of phages and satellites as extremely dynamic genetic platforms for the accumulation and diversification of bacterial immune systems.

KEYWORDS: Phage satellites, antiviral immunity, horizontal gene transfer, comparative genomics.

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Profiling the human virome landscape using Inviria

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Background. The human virome remains largely unexplored which limits our understanding of its role at ecological scale and subsequently in human health. Bulk and Virus-Like Particle (VLP) shotgun metagenomics have been adopted as reliable methods to study the viral component of the microbiome. However, current approaches mostly incorporate assembly-based workflows to resolve the viral composition and function *de novo*. These methods, albeit essential for revealing novel viral genomes, are not sensitive to low abundant viral species and often require heavy computational resources to run. Hence, we introduce Inviria, a computationally efficient pipeline that is able to capture low abundant viral signals from human bulk and viral-enriched metagenomic data by leveraging comprehensively curated human gut, oral and vaginal virome catalogues.

Methods. We developed Inviria to be an end-to-end lightweight pipeline from quality control to taxonomic profiles. First, we integrate viromeQC to determine the viral enrichment level of metagenomic sequencing reads by mapping them against prokaryotic markers. Next, reads are taxonomically profiled using sylph, a k-mer based classifier that estimates the containment average nucleotide identity (ANI) of reference genomes within a sample metagenome. This means that the unique k-mers for rare genomes are also detectable. Here, classification is performed against the Unified Human Gut Virome (UHGV) catalogue for the human gut virome, Human Oral Virome Database (HOVD) for the human oral virome and the Vaginal Microbial Genome Collection (VMGC) for the human vaginal virome. The output includes a visual summary of viral enrichment profiles along with a table detailing coverage metrics, viral taxonomy, predicted microbial hosts and inferred viral lifestyle for each viral genome.

Results. When benchmarked on mock datasets, Inviria demonstrated substantially lower runtimes and memory requirements compared to existing read-based virome analysis pipelines such as Phanta, Marker-MAGU, and BAQLaVa. Gut virome profiling of 10 healthy control bulk and VLP metagenomics samples displayed a trend towards higher viral richness in bulk samples showing its ability to capture intracellular and prophage-associated genomes in bulk data. Comparison of the gut virome composition between 29 healthy controls and 22 patients with IgA deficiency (IgAD), using assembly-based profiling and Inviria, revealed elevated proportions of similar viral families, such as Salasmaviridae, and bacterial hosts like Enterobacteriaceae in IgAD subjects. This demonstrates Inviria's concordance with assembly-based approaches in identifying altered viral signals from metagenomic data.

Conclusion. Inviria allows efficient characterization of the human virome by accurately profiling both bulk and viral-enriched datasets with reduced computational runtime and memory usage. Hence, this pipeline offers a scalable approach in exploring viral diversity and composition in human samples.

KEYWORDS : human virome, bioinformatics, metagenomics, diversity

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Determinants of phage resistance in clinical *Pseudomonas aeruginosa* strains

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The global rise of antibiotic resistance has renewed interest in phage therapy as a promising alternative. Yet, bacterial resistance to phages remains a major challenge, long attributed mainly to receptor modifications that block adsorption (Gaborieau *et al.*, 2024). Recent discoveries in bacterial immune systems suggest a broader picture (Georjon *et al.*, 2023): phages may adsorb efficiently but fail at later infection stages, with intracellular defenses, transcriptional regulators, or other factors acting as key determinants of susceptibility (Costa *et al.*, 2024).

To investigate this, we examined 125 clinical isolates of Pseudomonas aeruginosa from antibiotic treatment failure cases against 9 phages spanning multiple genera. Using high-throughput resistance assays, adsorption tests, genomic analyses, and genome-wide association studies (GWAS), we dissected the mechanisms underlying phage resistance. In the majority of cases, resistance was explained by a lack of adsorption. Nevertheless, we also found a significant negative correlation between the number of defense systems and phage infectivity, although considerable variation remains unexplained. Clustering analyses of infectivity profiles showed that susceptibility patterns are often shared across phages, with certain phages displaying highly similar host ranges. Finally, compositional analyses suggested that specific defense systems are associated with increased resistance.

These findings highlight both adsorption failure and intrinsic defenses as major determinants of phage resistance in clinically relevant isolates. Understanding how defense load and composition shape phage susceptibility is crucial for optimizing therapeutic applications and may help predict and circumvent bacterial resistance more effectively.

KEYWORDS: phage-host interaction, phage resistance, *Pseudomonas aeruginosa*, phage therapy

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Phage evolutionary relationships emerge from protein Language Model-based proteome representation

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Bacteriophages, viruses of bacteria, shape bacterial communities and offer promise to treat multidrug-resistant infections. Phage taxonomy is a daunting challenge due to their rapid evolution, frequent gene swapping, and ever-increasing volume of new genomes. We introduce HieVi (Hierarchical Viruses), a framework for comparative genomics of bacteriophages that leverages self-supervised protein Language Model (pLM) to generate a single vector representation for each phage proteome (Fig. 1). This "fingerprint" encodes functional and evolving information that enables the organization of phage genomes in accordance with existing taxonomy and facilitates the discovery of new taxa. HieVi framework constitutes a step towards scalable and searchable organisation of phage genomic data useful for refining existing taxonomy and exploring the intricate and ever-expanding landscape of the viral world.

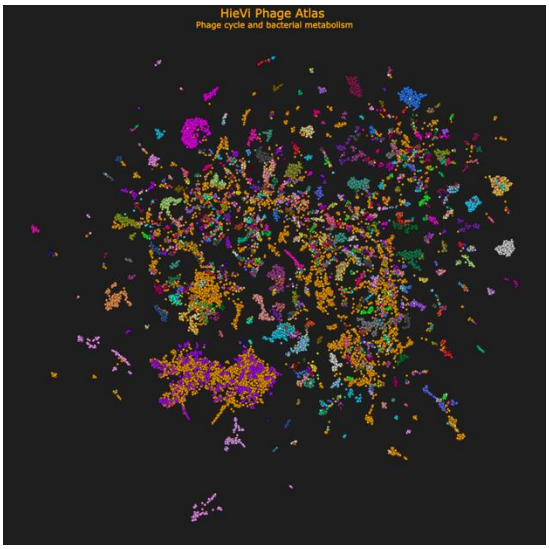

Fig. 1. HieVi Phage Atlas – UMAP 2D representation of 24,362 phage vectors generated with the HieVi pipeline (interactive map available at https://pswapnesh.github.io/HieVi/HieVi_UMAP.html)

KEYWORDS: Phage comparative genomics, phage taxonomy, protein Language Models, Artificial Intelligence.

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Evolutionary dynamics of phage domestication across prokaryotes

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Phages drive multiple functional innovations within microorganisms. It has been hypothesized that prophages were domesticated into contractile secretion systems driving inter-bacterial warfare, such as the Type VI secretion systems (T6SS). We setup to identify the complex processes of phage co-option and functional innovation leading to domesticated contractile systems across the available diversity of complete prokaryotic genomes.

To construct our dataset, we integrated different methods using sequence and structural similarity to search for distant homologs of experimentally characterized systems (tailocins, external contractile injection systems, T6SS). We detected more than 20,000 systems and related phages across bacteria and archaea, redefining their distribution. To reconstruct their most probable path of evolution, we build phylogenetic trees using a variety of techniques with tree search based on sequences (maximum likelihood and Bayesian sampling) or structures. Our results show that contractile systems have been co-opted from phages numerous times, particularly in the case of tailocins. We refined the phylogenies of each systems clade and identified common aspects between independent paths of prophage domestication. These results shed light on the long-term evolutionary impact of phages in prokaryotes.

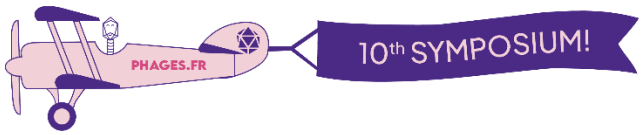

Fig. 1. Logo of the conference Phages in Nancy

KEYWORDS: Prophages, Domestication, Tailocin, T6SS, Co-options

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Diversity and prevalence of prophages and anti-phage defense systems in a major rice pathogen

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This work aims to address two main questions : How diverse are prophages and anti-phage defense systems across the phylogeny of a bacterial species? And, in the case of a widely distributed rice pathogen, how are these features distributed, and do they correlate with phylogeny or geographic origin ? *Xanthomonas oryzae* is a widely distributed bacterial species that poses a major threat to global rice production. Its diverse pathovars infect different host plants and tissues, with some causing devastating diseases such as bacterial leaf blight and bacterial leaf streak on rice, leading to significant yield losses on one of the world's most important staple crops. Moreover, the distinct lineages of *Xo* exhibit significant genetic differences. While the genomic architecture of *Xo* lineages is currently being investigated, the role of phage-related genomic features in shaping this diversity remains unexplored. Prophages and anti-phages defense systems are known to influence genome evolution, but their impact has mostly been studied in other bacterial taxa ; while such research on phytopathogenic bacteria remains scarce.

We analyzed prophage number, size, and diversity across 336 *Xo* genomes. In parallel, we investigated the number and distribution of defense systems to assess anti-phage strategies within and between populations. Most *Xo* genomes harbor one to two prophages, a pattern that is consistent across populations. In total, 426 prophages were identified, spanning 83 species. On the other hand, a total of 2,576 defense systems, encompassing 84 subtypes, were identified, with each *Xo* strain carrying between 4 and 14 defense systems, their number and types varying according to population.

Our results indicate a dichotomy in transmission dynamics of phage-related genomic elements within *Xo.* Prophage diversity does not follow the phylogeny of the host genomes but instead reflects the geographic origin of the strains, suggesting that prophages are acquired predominantly through horizontal transfer. In contrast, anti-phage defense systems exhibit high subtype specificity within each population and align with host phylogeny, consistent with stable vertical inheritance. Together, these findings provide the first systematic view of phage-related genomic features in *Xanthomonas oryzae*.

KEYWORDS: Defense system ; Prophages ; *Xanthomonas oryzae*

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Atypical replication error pattern and limited repair efficiency contribute to elevated mutation rate in phage Lambda

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Viruses exhibit higher mutation rates than their hosts, potentially enhancing their adaptability. Yet, the rates and spectra of mutations remain insufficiently characterized in double-stranded DNA (dsDNA) phages, despite their importance for understanding phage evolution and optimizing phage-based therapies. To address this, we used lambda, a model dsDNA phage that infects *Escherichia coli*, to (i) estimate its mutation rate, (ii) characterize its mutational spectrum and (iii) assess the role of host repair systems, particularly Mismatch Repair (MMR). We used four complementary approaches: mutation accumulation coupled with whole-genome sequencing (MA-WGS), duplex sequencing (DS), fluctuation assay (FA), and Mutation Visualization (MV), a microscopy-based error tracking method developed previously by our lab by Robert L. *and al.* in 2018.

MA-WGS and DS and FA yielded consistent results, estimating lambda mutation rate at ~5.10^{-9} per base per replication, ~15-fold lower than previously estimated by Drake J.W. in 1991 and ~20-fold higher than its host by Lee H. *et al*., in, 2012. DS and FA showed that lambda mutation rate increased only modestly (2-10x) in MMR-deficient host, compared with >100-fold increases observed in *E. coli* by Lee H. *et al*. in 2012. MV using fluorescently labeled MutL demonstrated that mismatches on the lambda genome are efficiently detected by the MMR system. We also found that the mutational spectrum of lambda grown on MMR-deficient hosts presents a marked enrichment in transversions compared to the *E. coli* MMR-deficient cells. This finding indicates that, although both genomes use the same DNA polymerase, they display distinct replication error profiles. As errors leading to transversions are less efficiently repaired by MMR, this likely accounts for the weaker MMR effect observed in lambda.

In conclusion, lambda mutates at ~5.10^{-9} per base per replication. The modest effect of MMR inactivation indicates host repair systems are partially effective, likely due lambda's distinctive error spectrum. These findings refine our understanding of phage evolution, highlight differences between phage and bacterial mutation processes, and provide methodological frameworks for future studies.

KEYWORDS: lambda, *E. coli*, Mutation rate, Mismatch Repair

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Host-virus dynamics in anaerobic digesters facing abiotic inhibition

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Anaerobic digestion (AD) is a process of great economic importance, but it is highly sensitive to disturbances. These disturbances inhibit the process and lead to a loss of methane production. Viruses are abundant and diverse within anaerobic digesters (Calusinska, M *et al*, 2016) and certainly play a major role in controlling the structure, dynamics and functions of AD microbial communities. A better understanding of these interactions is needed in order to fine-tune the process.

We studied the effects of various abiotic disturbances on the composition of the microbiomes and viromes, and on the performance of the process.

The diversity of prokaryotes and viruses was high, with Clostridiales dominating the prokaryotic community and *Caudoviricetes* dominating the viromes. We identified 132 viral contigs and 19 host genera that were differentially abundant under disturbed conditions, with a good agreement between the composition of prokaryotic community and predicted host taxonomy for viruses. Electron microscopy analysis revealed diverse virus-like particles in the AD, including head-tailed particles typical of *Caudoviricetes*, but also spherical, rod-shaped and spindle-shaped particles typical of archaeal viruses. Notably, we present a new virus family, *Eurekaviridae*, of spindle-shaped viruses associated with methanogenic archaea (Krupovic, M *et al*, 2025). No significant impact of the tested abiotic stresses on prophage induction was observed, suggesting that viruses had mild but continuous effects on host population dynamics rather than abrupt and devastating impact due to massive provirus induction. Numerous auxiliary metabolic genes were detected in the viral contigs, which could affect metabolic fluxes in the process. Our study further extends the knowledge on the diversity and dynamics of the AD microbiome and virome.

KEYWORDS: anaerobic digestion, viral ecology, methanogenesis, metagenomics, environmental biotechnology

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Decoding host-parasite encounters in soil bacteria through CRISPR-spacer footprinting and cell-surface genotype analysis

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The reconstruction of natural phage–host interactions in microbial communities is challenging due to their complexity and spatial heterogeneity. Moreover, almost nothing is known about the dynamics of bacterial CRISPR-Cas systems in the environment. We combined CRISPR-spacer "footprinting" with analyses of surface-exposed cell-envelope genes to infer parasite-host dynamics in *Myxococcus xanthus*, a cooperative soil bacterial predator that forms spatially structured fruiting bodies in soil.

Our study focused on >200 genomes collected across mm-to-km spatial scales within a focal soil landscape in Indiana (US), complemented by global isolates as phylogeographic references. Across all genomes, we annotated ~9,000 unique CRISPR spacers spanning four CRISPR-Cas systems and revealed broad phage-exposure histories and local spacer turnover. One of these systems, a type I-C CRISPR-Cas, is universally conserved, and we confirmed its activity through functional interference assays in a natural strain using targeted knock-out experiments.

Mining the population pangenome uncovered several inducible local mobile elements to which many local spacers match perfectly and indicate contemporary local infections. Among these elements is a prophage that resembles well-characterized *Myxococcus* phage Mx8, and another completely novel, plasmid-borne temperate phage. Strikingly, many spacers that previously lacked matches now align perfectly to the plasmid-borne phage, particularly in soil cores where the element occurs, but also at additional Indiana sites, expanding the recognised host-parasite network.

To complement CRISPR-based inferences, we analysed mutations in lipopolysaccharide (LPS) biosynthesis genes, which were previously identified as within-group selection hotspots (by Wielgoss et al., 2019). Notably, we discovered various mutations in these genes, which, combined with spacer acquisitions, represent half of all mutations observed in several networks. This suggests potential ecological or functional complementarity between cell-surface modification and adaptive immune memory among co-existing lineages within local microbial communities.

Together, our results show that the combination of spatially explicit sampling with reverse ecology, comparative genomics, and experimental validation has the power to reveal hidden layers of parasite-host interactions in complex environments like soil. By paying close attention to geographic distances and with the help of good isolation methods, we identified previously uncharacterized local phages and tracked their spread across microbial neighbourhoods. This integrative perspective highlights the promise of biogeography to shed light on microbial evolutionary biology and positions *M. xanthus* as a natural model system for deciphering the code of phage-host landscape coevolution.

KEYWORDS: CRISPR, phage biogeography, *Myxococcus xanthus*, reverse ecology

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Gut microbiome resilience promoted by phages following antibiotic treatment

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Antibiotic treatments induce perturbations in the gut microbiome by reducing bacterial diversity, which can have severe short- and long-term consequences (antibiotic-associated diarrhea, irreversible dysbiosis). Phages are numerous in the gut (10^8–10^9 per g feces), and are increasingly recognized as important modulators of gut microbiome diversity. Nonetheless, most recent research has focused on the impact of antibiotics on gut bacteria, overlooking phages. To address this gap, we recently re-analysed a phageome dataset obtained from 22 healthy individuals who received cephalosporin treatment for three days and were followed up to 180 days.

Globally, we found a decline of 20% in species richness after antibiotic administration that recovered within 30 days. Each individuum exhibited a distinct response, and this high individuality was retained post-treatment. Most strikingly, we observed a surge of virulent, highly-dominant phages the day after treatment that included phages infecting *Parabacteroides distasonis* (Pd). This gut commensal is resistant against many beta-lactam antibiotics and reported to thrive after cephalosporin treatment. Although, we detected Pd to bloom in one individuum, we found its phages more often to reach dominance (in three donors) in the absence of Pd. This suggests that Pd phages became dominant by preying on their hosts, when they were dominant, ultimately following Kill-the-Winner dynamics. We also suggest that the dominant phages are important in restoring gut diversity by preventing dominance of their bacterial hosts, and allow low-abundant species to recolonize the gut environment.

To test this, we aim to perform controlled bioreactor experiments using clinical-relevant antibiotics with different dosages to assess the generality of this response. Additionally, we will explore strategies to enhance microbiome resilience and recovery, including the use of food (dairy) derived phages.

KEYWORDS: metagenomic, phages, antibiotics, human gut, resilience

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Exploring the role of conjugative plasmid in the spread of phage resistance

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Mobile genetic elements (MGE), such as bacteriophages and plasmids, play a crucial role in horizontal gene transfer, notably promoting the spread of antimicrobial resistance genes within bacterial populations. However, MGE can also be detrimental to their bacterial hosts—and even lethal in the case of phages. To limit the intrusion of foreign DNA, bacteria possess an arsenal of defence systems, such as CRISPR-Cas and restriction–modification systems. Interestingly, these anti-MGE defence systems are often encoded by MGE themselves. For example, our preliminary data show that 60% of conjugative plasmids encode at least one anti-MGE system, suggesting that phages and plasmids strongly influence each other's dissemination within bacterial populations. Yet, the interplay between phage propagation and plasmid transmission remains poorly understood.
In this study, we focus on the dissemination of the conjugative F plasmid, which carries the abortive infection system Pif, in the presence or absence of phage T7. We aim to determine how these two MGE mutually influence each other's spread over short and long timescales. Specifically, we assess whether phage presence, at varying multiplicities of infection, inhibits or promotes the dissemination of the Fplasmid, and how this dynamic is affected by the Pif system.

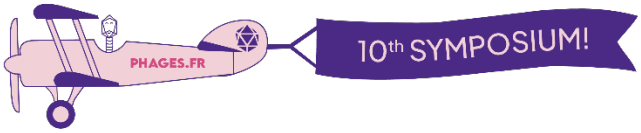

Fig. 1. Logo of the conference Phages in Nancy

KEYWORDS: Phage T7, Plasmid F, MGE competition, Defence system

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PHAGE-HOST INTERACTION

CELLULAR SCALE

The toxigenic conversion of *Vibrio cholerae* is favored by bacteriophage-encoded Xer activating factors

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Cholera is a major health issue worldwide. The ongoing 7th pandemic is caused by a single lineage of the bacterium *Vibrio cholerae*, which acquired its toxicity through lysogeny by the filamentous Cholera ToXin bacteriophage (CTXΦ). CTXΦ belongs to the IMEX family, which exploit the host Chromosome Dimer Resolution machinery, Xer, to integrate into the chromosomal Xer recombination site, *dif*. Another member of this family, the Toxin-Linked Cryptic (TLC) element has been consistently observed upstream of CTXΦ in clinical isolates. TLC does not encode any virulence factor, but its shared integration strategy with CTXΦ suggests it may play a role in the integration of CTXΦ during lysogenic conversion of *V. cholerae*. It was previously proposed that TLC facilitates CTXΦ integration by restoring functionality to a defective *dif* site. Our work demonstrates through *in vivo* and *in vitro* assays that the supposed defective variant is competent for Xer-mediated recombination, allowing Chromosome Dimer Resolution as well as CTX integration. Instead, we show that the Xer-activating factor of TLC (XafT), allowing its chromosomal integration, significantly favors the CTXΦ integration reaction. Furthermore, we identified a new Xer activating factor encoded by the VGJΦ bacteriophage, XafV, which also facilitates CTX integration. These results provide new information on the mode of action of Xaf proteins and reveal that different IMEXs have the ability to cooperate in the integration reaction. This mechanism appears as one of the drivers of toxigenic conversion in *V. cholerae*.

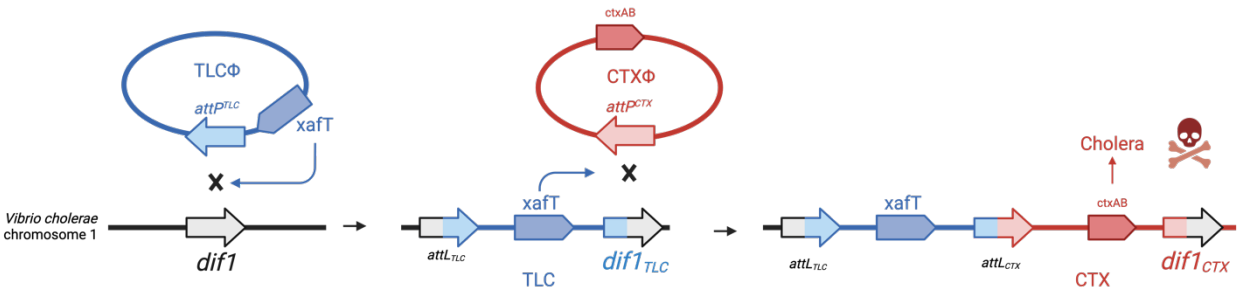

Fig. 1. Model for the role of TLC in CTX lysogeny in *V. cholerae* during toxigenic conversion

KEYWORDS: Cholera, Xer, Lysogeny, Recombination, Cooperation

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Tracking filamentous phage infection at the single-cell level

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Filamentous phages are non-lytic viruses that associate with a bacterial host in a mutualistic relationship. The acquisition of viral DNA by the host can promote genomic diversity, increase bacterial fitness and even enhance the virulence of certain bacterial pathogens that infect animals and plants. Successful symbiosis requires a coordinated sequence of events in which virion and host recognize each other, the phage particle is opened, and viral DNA is imported. However, the current model of filamentous phage infection remains incomplete and does not fully explain how the virus targets its host or crosses the bacterial envelope. Moreover, the spatiotemporal dynamics of this process are still poorly understood, because real-time visualization of phage infection had been very difficult until then.

The objective of my thesis project is to characterize in detail the infection process of the fd phage in its *E. coli* host using real-time fluorescence microscopy. The fluorescent tools developed by our team enable us to visualize the sequence of events, from the depolymerization of the phage capsid in the bacterial envelope to the replication of viral DNA in the host. In the future, the tools developed as part of the project could be applied to the study of other phage/host models of interest to animal or plant health.

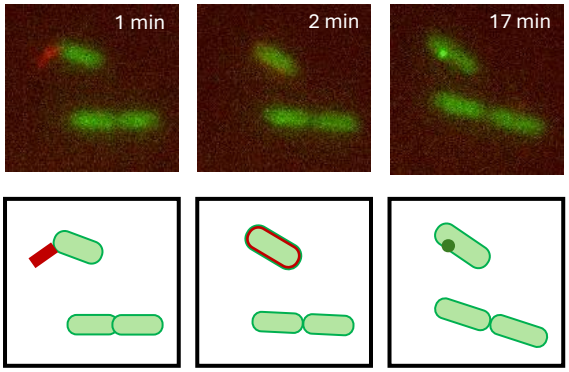

Fig. 1. Combined fluorescent tools for visualizing phage protein depolymerization and viral DNA replication

KEYWORDS: Filamentous phages, real-time infection, phage-host interaction, fluorescent microscopy

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2025 PHAGES IN NANCY

Exploring the functions of phage T5 pre-early genes in host takeover

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Upon infection of *Escherichia coli*, the virulent bacteriophage T5 employs an original two-step mechanism to transfer its 122-kb double-stranded DNA genome into the host cell. Initially, only 8% of the genome are injected, triggering a temporary pause in DNA transfer. This interval allows the expression of pre-early genes encoded in the first-step transfer (FST) DNA, which collectively reprogram the host cell and might neutralize its antiviral defenses before the remainder of the genome is delivered. A hallmark of T5's early infection phase is the rapid degradation of host DNA and nucleotide catabolism, processes mediated in part by the pre-early genes *A1* (a nuclease) and *dmp* (a dNMP nucleotide phosphatase). However, among the 17 proteins predicted to be encoded by the FST-DNA, the functions of eleven remain unknown.

In this study, we sought to elucidate the roles of these pre-early genes during T5 infection. Using a several approaches combining comparative genomics, structural modeling, reverse genetics, overexpression assays, fluorescence microscopy, and flow cytometry, we uncovered several insights into their functions. Through genome engineering (Ramirez-Chamorro et al. 2021), we demonstrated that only two pre-early genes, *A1* and *A2*, are essential for productive infection, while *dmp* and at least one additional gene enhance phage virulence. Strikingly, seven pre-early genes proved toxic when expressed in *E. coli* in the absence of other viral factors, inducing dramatic morphological alterations or nucleoid disorganization. While *A1* is the primary nuclease responsible for the rapid degradation of the host genome, at least two other pre-early genes are implicated in DNA processing. Additional observations suggest that some pre-early genes could have a function in modulating cell shape and disrupting membrane integrity.

Collectively, our findings provide a deeper understanding of how T5 manipulates its host during the critical early stages of infection, paving the way for further exploration of phage-host interactions and the molecular mechanisms involved in bacterial subversion by viruses.

KEYWORDS: Bacteriophage T5, pre-early genes, host takeover, DNA degradation

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Immunity, defence, anti-defence, anti-anti-defence... What else?

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Phages and bacteria have coevolved complex molecular systems over billions of years to ensure their survival. Bacteria have developed a wide variety of defense mechanisms, ranging from classic restriction-modification systems and CRISPR-Cas immunity to hundreds of recently discovered systems identified by genome and metagenome analyses. These defense systems often consist of sensor and effector components that detect phage infection and mount a specific response.

Phage escape mutants—phages that overcome bacterial defenses—help identify the molecular triggers sensed by bacterial systems. Frequently, these triggers are phage proteins, such as structural proteins or anti-defense factors like DNA mimic proteins (e.g., Ocr), which inhibit host restriction enzymes. One such defense, the PARIS system, detects DNA mimic proteins and blocks translation by cleaving host tRNAs. Beyond protein triggers, some systems may be activated by phage DNA or other nucleic acids structure formed during infection.

Older abortive infection systems like RexAB and P2 OLD, discovered in the 1950s and 1970s respectively, still lack well-defined triggers but are linked to responses against lambda phage replication. Both systems represent sophisticated, phage-dependent abortive infection defenses and highlight the long evolutionary arms race between phages and bacteria. Our recent study on P2 hotspot found in *E. coli* also identified Rex-like proteins that protect bacteria against P1 phage.

By deciphering the molecular mechanism of this system at biochemical level coupled to genetics, we hope to also find the triggering mechanism of the RexAB system.

KEYWORDS: Defence systems, DNA mimics, anti-defence, PARIS, RexAB

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Structure-function relationships between bacteriophage tail fibers and host range: investigations with *Nankovirus* and *Pakpunavirus* infecting *Pseudomonas aeruginosa*

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The adsorption of bacteriophages (phages) at the surface of bacteria is the first and a crucial step in infecting their host. It is mediated by specialized receptor-binding proteins (RBPs), such as tail fibers that recognize specific bacterial molecular motifs ie. host receptors. While the recognition between RBPs and host receptors is key for the definition of the phage host range, adsorption efficiency is a major determinant in the rate of infection.

In this study, we investigated the structure-function relationships between tail fibers and host range using two phages infecting the opportunistic pathogen *Pseudomonas aeruginosa.* Phage PAK_P4 (Pakpunavirus, 93 kb) isolated using strain PAK and phage CHA_P1 (Nankovirus, 88 kb) isolated using strain CHA), belong to two genera of the Skurskavirinae subfamily, share a nearly identical morphology but exhibit distinct adsorption kinetics. While 90% of CHA_P1 adsorbs to its host in 20 sec, it takes 5 minutes for 90% of PAK_P4 to adsorb to strain PAK. Moreover, the efficiency of plating (EOP) of phage PAK_P4 on strain CHA is 0.01, while the EOP of phage CHA_P1 on strain PAK is 0.3.

Taking advantage of the genetic proximity of these two phages we designed a genetic approach to study the role of the RBP domain in the fast adsorption of phage CHA_P1.

First we re-annotated phage genomes using *Pharokka* and identified three putative long tail fibers (LTF) encoding genes. Second, we employed *AlphaFold* predictions and the *Dali* server to refine the C-terminal regions of the LTFs likely involved in host recognition. Third, we constructed two recombinant phages in which the respective C-terminal domain of LTFs were swapped and characterized the resulting recombinant phages on strains PAK and CHA. PAK_P4 recombinant retained an EOP of 0.01 on strain CHA, while CHA_P1 recombinant showed only a slight increase to an EOP of 0.4 on strain PAK.

The adsorption of the PAK_P4 recombinant was not significantly affected on each strain, suggesting that the swapped domain either is not directly involved in the adsorption or that it has retained the molecular conformation required for adsorption on these two hosts. It also suggests that the fast adsorption of phage CHA_P1 is not strictly bound to this domain. In contrast, the CHA_P1 recombinant displayed a fast adsorption on both strains, showing that this property involves the swapped domain. These results suggest that beyond the swapped domain, conformational changes in the LTF could be involved in the fast adsorption phenotype. These data call for additional structural characterization of these recombinant proteins to identify the molecular determinants involved.

KEYWORDS: Adsorption, bacteriophage, One step growth

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Persistent virulent phages exist in bacterial isolates

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Despite the immense diversity of tailed bacteriophages, they are traditionally classified as either virulent or temperate, with only the latter thought capable

of long-term persistence in bacterial cells through lysogeny. Virulent phages, characterized by their obligatory lytic cycle, are assumed to lack the ability to persist within bacterial colonies, and their infection is expected to decimate the host population under *in-vitro* conditions. Consequently, when bacterial isolates are cultured for sequencing, the resulting assemblies are not expected to contain virulent phage genomes. To test this assumption on a large scale, we analyzed over 267,000 publicly available *Escherichia* assemblies. Surprisingly, we identified 373 genomes corresponding to virulent phages within

the bacterial genomes. These viral genomes are associated with specific phage groups and especially with jumbo phages with very large genomes (>200 kb). Chimallin was a core gene in two of these jumbo phage clusters, the major protein used by some jumbo phages to form a protective phage nucleus during infection. We found multiple lines of evidence suggesting that these virulent phage genomes in bacterial assemblies arise from persistent infections rather than contamination. Supporting this, we experimentally demonstrated the

coexistence of non-temperate jumbo phages with their bacterial hosts. In a targeted follow-up search for three clades of persistent jumbo phages, we

identified 285 additional jumbo phage genomes in bacterial taxa beyond *Escherichia,* highlighting that there are many more undiscovered persistent phages in bacterial assemblies. Our findings challenge the traditional virulent-temperate dichotomy, highlighting the overlooked diversity and prevalence of non-canonical phage lifestyles.

KEYWORDS: Pseudolysogeny, persistent infection, virulent phages

RÉFÉRENCES

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Explorations on the mechanisms that bacteria deploy to survive a bacteriophage cocktail challenge

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When bacteria are exposed to a single virulent bacteriophage, variants will escape this deadly predation. Previously, we showed that when the *Escherichia coli* strain 536 is challenged by the bacteriophage 536_P1 (Phapecoctavirus, 149 kb) most of the mutations identified in resistant clones were located on pathways involved either in LPS or capsule biosynthesis (Gaborieau et al.). Here, we questioned how the *E. coli* strain 536ΔPAI3, a variant of strain 536 lacking a pathogenicity island, responds to a challenge by a cocktail of 5 bacteriophages, among which two are unable to infect this strain. We performed the challenge in a large set of conditions, including planktonic- and biofilm-grown cells as well as persister cells obtained after exposure to an antibiotic. Moreover, we utilized different bacteriophage:bacteria ratios and exposure times. This broad variety of experimental conditions was chosen to mimic the heterogeneity of clinical contexts where this cocktail could be applied.
Surviving bacteria from all conditions were first frozen at the end of experiments and later spread on plates in absence of bacteriophages. To explore the diversity of putative resistance mechanisms, we used a picking robot that collected randomly about 100 clones per condition. Next, we tested the susceptibility of 2791 clones to the 5 individual bacteriophages and the cocktail. Phenotypes were grouped by profiles of susceptibility/resistance and revealed that the proportion of resistant clones increases with the dose of bacteriophages and the contact time. However, when performing a second test several months later, a large proportion of clones displayed a different phenotype demonstrating that the initial phenotypic resistance was unstable. Among these clones, we selected 21 for genome sequencing. We identified 9 candidate genes and performed genetic complementation to confirm their involvement in bacteriophage resistance mechanisms.
Beyond genes likely coding for bacteriophage receptors, we also identified genes encoding metabolic enzymes and regulatory proteins. These results revealed that, beyond mutations of receptors, bacteria must develop alternative or complementary solutions to overcome the challenge imposed by a cocktail of 5 bacteriophages.

KEYWORDS: Bacteriophage, *Escherichia coli*, Phenotypic heterogeneity, Resistance

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Interspecies competition between *Citrobacter rodentium* and *Escherichia coli* involves prophages

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Prophages are known to mediate the transfer of their DNA from one bacterium to another. This is occurring frequently within bacterial populations such as those inhabiting the digestive tract of mammals. This process has been studied extensively in the laboratory through the lens of pathogens that carry prophages encoding toxins. Much less is known about the conditions and the consequences of prophage induction in the intestinal microbiota.

Citrobacter rodentium is a murine enteric pathogen harboring 10 prophages among which two (ϕNP and ϕSM) are constitutively induced and are known to infect the *Escherichia coli* strain MG1655. We leveraged this system to investigate the possible consequences of induction of these two prophages on *E. coli*. *In vitro* co-culture experiments of *E. coli* with either wild-type or prophage-less mutant of *C. rodentium* demonstrated that ϕNP is able to lysogenize *E. coli*. Therefore, we observed a minimal direct competition between the two species, suggesting that the mechanism of lysogenic conversion may attenuate the apparent antagonism of *C. rodentium*. *In vivo*, we first colonize gnotobiotic mice OMM^{12} with the *E. coli* strain for 7 days before to administer *C. rodentium*. During the onset of the infection by *C. rodentium* and for 12 days, we observed no variation in the intestinal *E. coli* load. However, from day 12 to day 20 the *E. coli* load decreased progressively and then stabilizes, suggesting lysis followed by lysis-then-lysogeny dynamic. These findings revealed insights into the role of temperate phages as ecological modifiers of microbial communities in the gut.

KEYWORDS: *Citrobacter rodentium*, *E. coli* MG1655, Prophages, Lysogen, OMM^{12} mice.

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From mouse to man: building models to quantify the dynamic interactions between bacteriophages and bacteria on the pulmonary epithelium

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The Gram-negative pathogen *Pseudomonas aeruginosa* is a leading cause of pneumonia worldwide and is known for its resistance to multiple antibiotics. Bacteriophages of *P. aeruginosa* have been shown to treat efficiently pulmonary infections in mice and have been used for compassionate treatments in Europe and in the USA. However, the mechanisms underlying therapeutic success and in particular the impact of phage amplification in organs remain poorly studied.
Here, we established a two-tier modeling strategy using human primary alveolar epithelial cells (HPAECs) and human primary lung microvascular endothelial cells (HVMECs-L): (i) a transwell model developed by Fonseca et al. and (ii) a lung-on-chip platform. In the transwell model, HPAECs were cultured at an air-liquid interface (ALI) for 14 days. Immunostaining revealed that HPAECs expressed alveolar type 1 (AT1) and type 2 (AT2). Transepithelial electrical resistance measured on ALI day 14 and the detection of the tight-junction protein ZO-1 indicated an intact epithelial barrier. Following the addition of *P. aeruginosa* strain PAK-GFP we observed a rapid growth of bacteria and revealed that this strain is able to invade epithelial cells. In the lung-on-chip platform, HPAECs and HVMECs-L can grow and differentiate in the chip, upon exposure to ALI day 14 and in presence of a mechanical force to mimic breathing. Immunostaining confirmed epithelial differentiation with detection of AT1 and AT2 pneumocytes. In addition, a first attempt to infect these chips with *P. aeruginosa* showed that bacteria can rapidly grow and attach to the surface of epithelial cells (4h and 24h p.i.) under the dynamic flow of media and in the presence of the mechanical force. We also introduced the phage PAK_P4 and found that it was capable of reducing the bacteria load in the chip within 4h.
These preliminary data showed that HPAECs can differentiate into AT1 and AT2 cells when cultivated in the ALI in both systems. Experimental conditions are being refined to allow the dynamic recording and quantification of bacteriophage infection at the cellular level in both models.

KEYWORDS: human primary alveolar epithelial cells, transwell model, lung-on-chip, *Pseudomonas aeruginosa*, phage treatment

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Production de phages par synthèse acellulaire

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Les bactériophages offrent une perspective prometteuse pour contrer la montée des résistances aux antibiotiques dans le traitement des infections bactériennes. Cependant, un des principaux inconvénients de leur utilisation réside dans la dépendance de leur production à l'égard de leur hôte bactérien, un pathogène, souvent virulent et parfois antibiorésistant. Cette manipulation présente des risques significatifs pour la santé des manipulateurs et des risques de contamination en dehors des environnements contrôlés. De plus, la préparation d'une suspension thérapeutique nécessite l'élimination rigoureuse de tout hôte vivant résiduel et des débris bactériens, qui peuvent induire des réactions inflammatoires.

Ainsi, il est très avantageux d'être en mesure de produire des phages sans leur hôte bactérien. Notre objectif est d'adapter la technologie acellulaire (*cell-free*) à la production de phages. Cette technologie permet de créer des milieux réactionnels à base de lysats cellulaires capables de transcrire et de traduire l'ADN. Actuellement, elle est principalement utilisée pour la synthèse de protéines et de particules virales (Virus-Like Particles, VLP), mais elle peut être adaptée pour produire des phages infectieux (Emslander *et al*. 2022).

Dans le cadre de nos recherches, nous avons réussi à produire le coliphage T7 en utilisant le kit *cell-free* SynXpress fourni par l'entreprise Synthelis. Nous nous efforçons désormais d'améliorer le titre infectieux obtenu et d'explorer d'autres candidats viraux pour la synthèse. A terme, nous espérons synthétiser des phages présentant un intérêt thérapeutique, tels que ceux ciblant des bactéries gram négatif du genre *Vibrio ou Yersinia*.

KEYWORDS: phages, phagothérapie, bioproduction, acellulaire, biologie synthétique

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Systematic functional assessment of anti-phage systems in their native host

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Bacteria deploy diverse anti-phage strategies, yet their functionality in native hosts remains poorly understood. Here, we investigated eight predicted anti-phage systems in the clinical *Escherichia coli* isolate NILS69 against 93 virulent phages. Phage resistance was mainly explained by adsorption barriers, while only a restriction–modification system and PD-T4-3 provided detectable protection. The restriction-modification system acted via a predicted type IV endonuclease and was also able to limit plasmid conjugation. Other defence systems showed no detectable activity, likely owing to phage specificity, environmental regulation or cofactor requirements. These findings underscore the need for further studies to investigate the regulation and ecological roles of bacterial defence systems in their native host contexts.

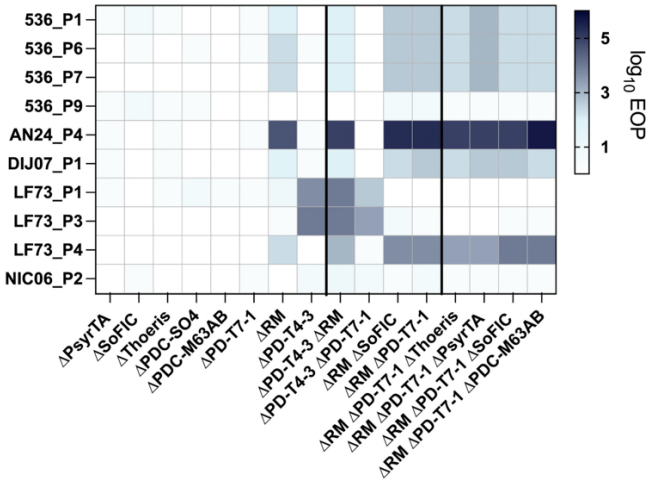

Fig. 1. Phage infectivity profiles against defence systems mutants of *E. coli NILS69*.

KEYWORDS: bacterial immunity, defence systems, phage, *Escherichia coli*

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STRUCTURE AND ASSEMBLY

MOLECULAR SCALE

The cryoEM structure of the *Mycobacterium abscessus* phage Jabs reveals a unique host-binding machinery

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Phage host-binding machineries, located at the tail tip, recognize host-specific receptors and initiate infection. At the 2023 Phages in Lyon meeting, we reported the first AlphaFold2-based structural information on the proteins assembling the host-binding machineries of phages infecting mycobacteria (mycophages), underlining their originality compared with those of phages infecting canonical Gram+ and Gram- bacteria (by Cambillau, C & Goulet, A in 2023). Here, we present the cryoEM structure of the mycophage Jabs, which reveals a unique architecture of the host-binding machinery likely adapted to the complex mycobacterial cell wall (not published). Notably, Jabs is the first mycophage shown to preferentially infect the smooth morphotype (i.e., with glycopeptidolipids in the cell wall) of the human pathogen *Mycobacterium abscessus* (Mabs) (by Liew JH, under review), whereas all previously described Mabs phages, including those used for therapy (by Dedrick, RM in 2019), target the rough morphotype (i.e., without glycopeptidolipids in the cell wall). Comparative structural analyses of Jabs and rough-specific Mabs phages will shed light on the molecular diversity of phage–mycobacteria interactions. These findings will also provide a structural framework for phage therapy, as both morphotypes coexist in infections, with the smooth form prevailing in some patients (by Liew JH, under review).

KEYWORDS: phage-host interactions, host-binding machinery, cryoEM structure, mycobacteria

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The bacteriophage SPP1 connector reopening is triggered by the tail binding

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Tailed bacteriophages share a particularly complex organization. Their double-stranded DNA genome is held under high pressure inside an icosahedral capsid. The connector, a channel allowing genome entry and exit during the viral cycle, occupies one of the twelve vertices of the capsid. This connector is also the binding site for the bacteriophage tail. During the past years, the high-resolution structures of a growing number of bacteriophages with tails have been determined. However, the detailed pathways leading to the assembly of these particles remains to be elucidated.

The SPP1 bacteriophage is a particularly interesting model system given the extensive corpus of genetic and biochemical data on the various structural proteins and their incorporation during viral particle assembly and infection. A previously determined reconstruction of the isolated connector of SPP1 had provided clues about the closure mechanism for this virus. However, the observed structure was markedly different from the ones observed in closely related bacteriophages.

To understand this difference, we determined reconstructions of the SPP1 connector inside the mature phages and inside the nucleocapsid, the last assembly intermediate before recruitment of the viral tail. A significant conformational change of the head closure protein was observed upon tail binding. Comparison of these structures have enabled us to propose a two-steps mechanism for keeping the viral genome inside the capsid.

KEYWORDS: SPP1, cryoelectron microscopy, phage assembly, DNA retention, head-tail junction

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26:7283

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Structural characterization of a sugar-binding siphophage

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Siphoviruses exhibit at the tip of their tail a macromolecular complex called the baseplate. This complex is known to encompass receptors that can specifically interact with the bacterial host (e.g., with proteins or carbohydrates). This reaction triggers phage infection. Recently, a growing number of structural studies of siphophages reveal the wide diversity of their baseplate architecture. However, little is known about the structural rearrangements of the baseplate upon host recognition and infection, except for T5, Lambda and Bxb1 siphoviruses.

As shown for T5 and Lambda siphoviruses, phage-host interactions lead to dramatic conformational changes: the tip's central fiber bends, brings the phage tail closer to the host, allowing for membrane perforation and DNA ejection. While T5 and Lambda are known to bind protein receptors of E. coli (FhuA and LamB, respectively) through the tip of their central straight fibre, the molecular mechanisms of infection of siphoviruses that recognize carbohydrate motifs on Gram-negative bacteria, through multiple spikes, remain unknown (Figure 1).

Our study focuses on 9NA siphovirus, which receptor is the LPS of Salmonella. We obtained a complete structure of the native virion by single particle cryo-EM. 9NA exhibits a baseplate with a central trimeric nozzle and six surrounding spikes, as described for lactophages. To study the infecting state, we used purified LPS or solubilized bacterial membrane into nanodiscs to trigger 9NA tail tip opening and we plan to acquire single particle cryo-EM data. To get a step closer to the in vivo mechanism, we infected Salmonella mini-cells with 9NA and collected cryo-electron tomography data. Early results of tomogram inspection and subtomogram averaging show at low resolution how the baseplate stick to the cell at different stages of infection.

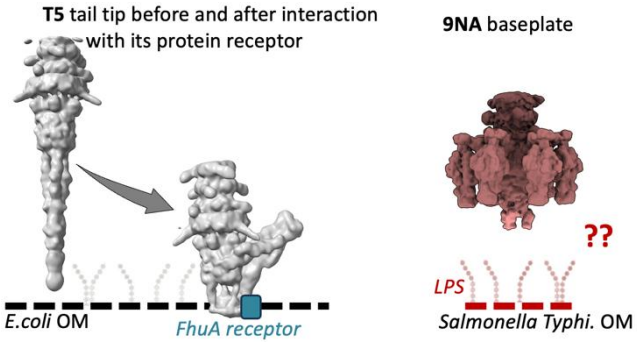

Fig. 1. Structural changes of a siphoviral baseplate after interaction with sugar motifs are uncharted.

KEYWORDS: Siphovirus ; 9NA ; Cryo-EM ; structure ; baseplate ; tail ; LPS

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SOCIAL SCIENCES

Alteration of Gut Phageome in Patients with Multiple Sclerosis

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Background. Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS) that potentially causes severe neurological disability. While the gut bacteriome has been demonstrated to be an environmental factor with a coercive impact in the pathogenesis of MS, possible alterations of the gut phageome before and after MS diagnosis remains to be characterized. The aim of this study was to dissect gut phageome composition that discriminates between healthy subjects and patients with first onset of MS (Clinically Isolated Syndrome ; CIS) and chronic MS disease.

Methods. To elucidate the composition of gut phageome in different MS clinical phenotypes, 42 MS patients, 12 patients with newly diagnosed CIS and 25 healthy individuals were enrolled in our study. Free viral particles from stool were filtered and concentrated, and non-encapsidated DNA fragments were enzymatically removed, after which viral DNA was purified and sequenced on Illumina. In parallel we also deep shotgun sequenced total metagenomic DNA so as to compare phage and bacterial dynamics. Following sequence assembly, viral contigs were identified using VirSorter2, DeepVirFinder, geNomad and VIBRANT. The quality of predicted viral contigs was then assesed using CheckV and the contigs were taxonomically annotated using IPHOP. Viral contig identification, host prediction and coverage estimation allowed us to study the phageome composition, diversity and functions.

Results. We identified 13,250 viral contigs that were clustered into 5,220 unique viral Operational Taxonomic Units (vOTUs). The sequence size distribution of vOTUs inlucded 423 high-genome-length vOTUs (>50 kb), 2,473 medium-sized vOTUs (10-50 kb) and 10,354 with low-genome-length size vOTUs (< 10 kb). 1,238 vOTUs were annotated as medium and high-quality with 796 vOTUs representing complete phage genomes. Comparative analysis revealed a decrease in phages targeting *Alistipes* and *Oxalobacter* in CIS patients compared to both healthy subjects and MS indicating specific phageome alterations before chronic MS disease. We also observed a gradual reduction in phages targeting *Prevotella* in MS patients relative *to* healthy controls. This is consistent with previous studies that have reported decreased abundance of *Prevotella* in patients with MS, suggesting that phage community composition is shaped by the dynamics of their bacterial hosts.

Conclusion. Our findings shed lights on distinct gut phageome alterations even before chronic MS disease sets in.

KEYWORDS : multiple sclerosis, virome, phageome, metagenomics,

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APPLICATIONS IN THERAPY AND BIOTECHNOLOGIES

ALPHAGOS: AN AI-DESIGNED PHAGE COCKTAIL FOR CONTROLLING *ENTEROCOCCUS CECORUM* IN BROILER CHICKENS

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Addressing the urgent need for antibiotic alternatives in poultry production, we developed *Alphagos*, a two-phage cocktail designed with the AI-powered tool CocktailPHinder. This tool enabled the selection of phages active against five representative *Enterococcus cecorum* strains isolated from broiler farms. In collaboration with our partners, we evaluated *Alphagos* in vivo in 65,000 heavy male broilers.

During a 36-day trial, the cocktail was administered via drinking water in nine doses (10^6 PFU/animal). Phages were consistently detected in water, feces, and organs, persisting up to seven days post-treatment. Despite elevated early mortality linked to chick quality and concurrent *E. coli* infection, no antibiotics were required, and phage colonization was confirmed.

These findings demonstrate that *Alphagos* can limit *E. cecorum* colonization under field conditions and highlight AI-guided phage design as a scalable and practical alternative to antibiotics in poultry production.

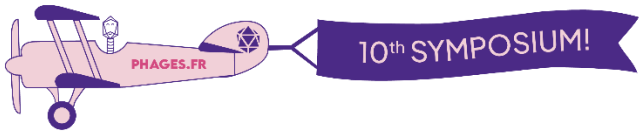

Fig. 1. Logo of the conference Phages in Nancy

KEYWORDS: Phage cocktail, *Enterococcus Cecorum*, poultry, antibiotic alternatives, AI design

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Interaction between bacteriophages and innate immunity as a determinant for the efficacy of phage therapy

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The rise of antimicrobial resistance has led to renewed interest in evaluating bacteriophages (phages) as a therapeutic alternative, although the success of phage therapy is influenced by a multiplicity of factors, not all of which have been thoroughly investigated. Among these factors, the host immune response to the treatment is likely to play a pivotal role. Here we use a murine model of pulmonary infection with *Pseudomonas aeruginosa* to study the impact of innate immune cell populations, namely neutrophils and alveolar macrophages (AM).

Roach et al., in 2017 showed that the same phage treatment regimen against *P. aeruginosa* pneumonia that had proven effective in immunocompetent mice, failed to resolve infection when the animals were immunocompromised, whether due to neutropenia or innate immune-signaling deficiencies. To further understand conditions under which neutropenia would render phage therapy ineffective, we identify through mathematical predictions a potential regime for intermediate neutrophil depletion. This would lead to a therapeutic outcome that does not fully correspond neither with immunocompetent nor immunodeficient mice, but rather with the inherent bistability of *in vivo* dynamics. These predictions were then confirmed by showing that *in vivo* phage therapy rescue a subset of individuals in intermediate depletion regimes, depending on the phage type. Subsequently, in Zborowsky et al. (2025), we evaluate the role of AM in the same infection model and show that, unexpectedly, AM-depleted mice had a better outcome of phage treatment, compared to immunocompetent animals. Simulations from a mathematical model of phage, bacteria, and innate immune system dynamics, suggested that this is likely due to AM reducing phage density in the lungs. This was experimentally confirmed with *in vivo* data showing faster decay of phage population in immune-competent mice compared to AM-depleted animals, as well as *in vitro* data showing AM phagocytosis of therapeutic phage.

These findings demonstrate the involvement of feedback between phage, bacteria, and the immune system in shaping the outcomes of phage therapy in clinical settings and highlight the critical need to assess host status, particularly immune function, as inclusion criteria for phage therapy.

KEYWORDS: Innate immunity, antimicrobial resistance, phage therapy, neutrophil, macrophage

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The potential of phage endolysins in wine preservation

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Grapes are one of the most valuable crops, with 15.9 billion litres of wine being produced in the European Union alone. To transform grapes into wine, two rounds of fermentation take place. First an alcoholic fermentation turning sugars into alcohol, followed by malolactic acid fermentation by lactic acid bacteria. Where several LAB species associated with wine, especially Oenococcus oeni, have a positive impact to wine sensory characteristics, other members of the LAB community, such as Lentilactobacillus spp. and Pediococcus spp., can produce undesirable compounds (Tempère et al., 2018). Most of the measures that are in place to control spoilers, rely on chemical or physical treatment of the wine (Stockley et al., 2021). The demand for more natural alternatives by consumers and environmental factors made us examine the potential of phage endolysins to be applied in wine as a biological control for undesired bacteria. Endolysin sequences have been extracted from both public and private sequences of lytic and temperate phages infecting O. oeni and Lentilactobacillus spp. Using an in silico approach, the endolysins have been classified and candidates were selected for cloning and production, after which their bactericidal activity is evaluated.

KEYWORDS: phage, endolysin, wine, *Oenococcus oeni*

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Design of phage-based hydrogels as innovative strategy for the treatment of severe acne by phage therapy

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Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit, affecting adolescents and young adults worldwide. The disease is multifactorial, involving hormonal regulation, sebum production, inflammation, and the proliferation of the commensal bacterium *Cutibacterium acnes*. Current treatments rely largely on topical and systemic antibiotics, retinoids, and hormonal therapy. However, the long-term efficacy of these approaches is increasingly limited by the global rise of antimicrobial resistance, frequent relapses, and significant adverse effects. The use of bacteriophages targeting *C. acnes*, including antibiotic-resistant strains, represents a promising alternative but developing smart phage delivery systems are still required. In this context, hydrogels are good candidates as they allow localized and controlled application directly onto the skin, thereby improving therapeutic efficacy while minimizing systemic side effects. Hydrogels composed of poly-L-lysine hydrochloride and hyaluronic acid are of special interest because of their favourable polyelectrolytic properties, which are expected to support phage stability, preserve infectivity, and enable controlled release at the site of infection.

In this work, a library of clinical *C. acnes* isolates was established to capture the genetic and phylotypic diversity of the species. In parallel, lytic bacteriophages targeting these strains were successfully isolated from wastewater samples. These phages are presently subjected to detailed characterisation, including morphological analysis by atomic force microscopy, host range evaluation, and genomic profiling to assess diversity and potential lysogenic traits. In the next phase, selected phages will be incorporated into hydrogels composed of poly-L-lysine hydrochloride and hyaluronic acid. The formulations will be evaluated for their ability to maintain phage viability over extended storage periods and for their antibacterial efficacy *in vitro*.

In conclusion, this project aims to demonstrate the potential of phage-based hydrogels as an innovative topical strategy for acne vulgaris. This approach could bypass the limitations of conventional treatments and pave the way for new perspectives in the management of chronic skin infections.

KEYWORDS: Acne vulgaris, *Cutibacterium acnes*, Bacteriophages, Hydrogels, Phage therapy

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Dissemination of antibiotic resistance by environmental bacteriophages in a constructed wetland

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The use of constructed wetlands (CWs), a nature-based solution, in wastewater treatment is increasing. These structures aim to improve the physical and chemical quality of effluents from Wastewater Treatment Plant (WWTP) before they are released into the environment (Maurice, 2022). However, WWTPs are not designed to remove antibiotic resistant bacteria, antibiotic resistance genes (ARGs), Mobile Genetic Elements (MGEs), or bacteriophages and their fate while flowing through CW remains poorly understood. High concentrations of bacterial ARGs and MGEs can be found in natural phage capsids due to their accidental capture during capsid assembly (Sagrillo, 2022). This phenomenon may enable environmental bacteriophages to disseminate antibiotic resistance through horizontal gene transfer (transduction) in ways that are not yet understood.

Our project aims to study antibiotic resistance and its dissemination by bacteria and phages in a large pilot CW (6 ha) consisting of three parallel ponds planted with different types of vegetation. DNA has been extracted from the bacteria and phages of water samples collected at various points of the WWTP and around the CW. Quantitative PCR have been performed to quantify 16S rDNA, 3 ARGs (*aadA*, *aadB* & *aacA4*) and 1 MGE (class 1 integron). These quantifications enable us to compare the relative abundances of different antibiotic resistance markers in bacteria and phages and to determine whether changes in these abundances occur as water passes through the CW. In parallel, a sequencing analysis has been performed on total DNA to assess the local phage ecology and to determine whether there has been a shift in the viral communities in the CW.

Taxonomic classification shows little change in bacteriophage communities from the upstream to the downstream part of the WWTP/CW ecosystem. Nevertheless, metagenomic analyses on the *16Sr DNA* extracted from the capsids revealed *Paenibacillaceae* & *Bacillaceae* to be the main phages releasing bacteria in the WWTP entry, while *Burkholderiaceae* & *Propionibacteriaceae* were predominant at the CW exits. This demonstrates a shift in the transducing phage species between the entry and the exit of the ecosystem. Moreover, the abundances of antibiotic resistance markers (relative to 16S rDNA and/or class 1 integrons) in bacteria tend to exhibit gradients from upstream to downstream within the ecosystem. Analyzing such abundances in DNA from the viral fraction remains tricky. Therefore, it is still difficult to conclude on the efficiency of the WWTP/CW ecosystem in reducing antibiotic resistance abundances and mitigating its dissemination is still difficult and further analyses and studies are needed.

KEYWORDS: Constructed wetland, Antibiotic Resistance Genes, Mobile Genetic Elements, DNA encapsidation

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Enhanced killing of multidrug-resistant Enterococcus faecium by Porthos phage–daptomycin combination therapy

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Enterococcus faecium is an opportunistic pathogen that frequently causes hospital-acquired infections and exhibits extensive intrinsic and acquired resistance to multiple antibiotics, including vancomycin. As conventional therapies have become increasingly ineffective, combination therapy incorporating bacteriophages with antibiotics has emerged as a promising approach to combat multidrug-resistant *E. faecium.* Among the virulent phages isolated, Porthos demonstrated the broadest host range and highest replication efficiency, by Lossouarn, J. in 2024. To evaluate the therapeutic potential of phage–antibiotic combinations, we systematically examined the interactions between Porthos and clinically relevant antibiotics using both simultaneous and sequential treatment models. Daptomycin exhibited the strongest synergistic activity with Porthos across both models, resulting in markedly enhanced bacterial clearance compared with other antibiotic combinations. Sequential treatment, in which bacterial cultures were pre-infected with the phage prior to antibiotic exposure, produced the most pronounced synergy. When combined with cell wall– or membrane-targeting antibiotics such as daptomycin, oritavancin, teicoplanin, and telavancin, Porthos markedly enhanced bacterial killing. This enhanced efficacy likely results from reciprocal effects on cell envelope integrity—phage-induced perturbations that increase antibiotic access and antibiotic-mediated membrane disruption that facilitates phage propagation. In contrast, protein synthesis inhibitors such as tigecycline and tedizolid showed weak or antagonistic interactions, likely due to inhibition of the bacterial translation machinery required for phage replication. To translate these findings *in vivo*, we are developing a zebrafish infection model using GFP-expressing *E. faecium* and *cftr*-deficient zebrafish to identify optimal phage–antibiotic combinations with therapeutic potential against multidrug-resistant enterococcal infections.

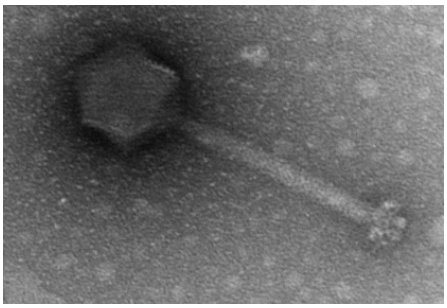

Fig. 1. Phage Porthos portrait

KEYWORDS: *Enterococcus faecium*; bacteriophage therapy; phage–antibiotic combination; multidrug resistance; zebrafish infection model

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