microLife, 2025, **6**, uqaf001
DOI: 10.1093/femsml/uqaf001
Advance access publication date: 31 January 2025
Commentary

"French Phage Network" annual conference—eighth meeting report

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Editor: [Carmen Buchrieser]

Abstract

The French Phage Network organizes a scientific meeting every year in which the community of researchers from academia and industry, as well as clinicians participate due to the growing interest in phage therapy. Although centered on giving exposure to future generations of scientists from the French community with senior investigators invited as main speakers, the meeting has also welcomed participants from other countries. Covering almost every aspect of bacteriophage biology, the meeting is an opportunity not only to expose the youngest to a broad range of topics, but also to share their most recent "work in progress" without undergoing a stringent selection process to obtain an oral presentation slot. This report reflects the dynamism of the research field on bacteriophages across multiple disciplines, including molecular and structural biology, ecology, evolution, therapy, and biotechnology.

Keywords: bacteriophage; phage-host interactions; ecology; evolution; structural biology; phage applications

Introduction

From 27 to 29 November 2023, the network of teams studying bacteriophages in France organized its eight annual meeting in the Domaine de la Croix Saint Joseph near Lyon. This all-inclusive venue selected by the local organizers provided a highly favorable environment for intense scientific exchanges. As usual, the meeting was organized around four sessions of talks, each introduced by a guest speaker. Two poster sessions stimulated vivid discussions. This meeting report, written by young members of the network (PhDs and Postdoctorate Fellows), provides a snapshot of the data presented during each session: ecology and evolution, structure and assembly, phage and host interactions, and therapy and biotechnology.

Summary of the scientific sessions

Ecology and evolution

The conference opened with a keynote presentation by **Jakob Træland Rostøl** (Postdoctoral Fellow, Centre for Bacterial Resistance Biology, Imperial College London, United Kingdom), who explored how prophages can protect bacteria from phage infection and could then be considered as being part of the bacterial immune system. In particular, he highlighted the Tha [tail-activated, higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domain-containing antiphage system] defense system used by *Staphylococcus aureus* prophages such as the 80α temperate phage (Rostøl et al. 2024).

During the prophage stage, when a new *S. aureus* phage infects, the newly produced minor tail proteins trigger the activation of the nonspecific RNase activity of Tha. This activation inhibits phage replication, thereby protecting the host cell. However, during prophage induction, Tha expression decreases due to reduced c1 promoter activity, and at the same time there is an increase in production of the Ith protein, which inhibits Tha. This regulation allows 80a to complete its lytic cycle and produce the minor tail protein without causing autoimmunity. As a result, the carried Tha protein protects both the prophage and its bacterial host from phage predation and is under tight regulation to allow the induced prophage to evade this immune system.

This original mechanism of action of the Tha protein illustrates the complexity of antiphage systems ruling phage–bacteria interactions. **Hugo Vaysset** (PhD student, Institut Pasteur, Paris,

Received 11 November 2024; **revised** 2 January 2025; **accepted** 16 January 2025

France) aims to unravel and predict these phage–bacteria interactions using genome analysis and algorithmic methods. Using 403 phylogenetically diverse *Escherichia coli* strains and 96 bacteriophages from 19 viral genera, he studied their interactions at three phages: bacteria ratios and in triplicates, which represents a matrix of 38 688 tests. The genomic features involved in the phage host range were characterized and receptor binding proteins (RBPs) were identified as a hotspot of genomic variability (Gaborieau et al. 2024). Findings indicated that the adsorption factors are strongly associated with the strain from which a given phage was isolated. Notably, no correlation was found between the number of bacterial defense systems and infection rates, although an increase in defense systems was associated with reduced virulence of infecting phages. In addition, he presented an algorithm for designing tailored phage cocktails to target pathogenic *E. coli* from pulmonary infections. Among 18 promising predicted cocktails tested on a collection of 100 strains, three generic cocktails have lower performance compared to specified tailored phage cocktails. Overall, this study provides new quantitative insights into phage–bacteria interactions and demonstrates the potential utility of integrating predictive algorithms into phage therapy.

Marion Saunier (PhD student, I2BC, Gif-sur-Yvette, France, and University of Sherbrooke, Canada) investigated a potential abortive infection (Abi) system in *Clostridioides difficile*, a major human pathogen linked to antibiotic-associated diarrhea. They identified a noncoding RNA (ncRNA) associated with the putative Abi system, located in a prophage conserved in epidemic ribotype 027 strain of *C. difficile*. They unraveled the regulation mechanism of the Abi-like system by the ncRNA, suggesting a mechanism similar to type III toxin–antitoxin systems. The Abi-like system may play a role in phage defense and stress response, as well as prophage stabilization in *C. difficile* (Saunier et al. 2024).

Chloé Feltin (PhD student, INRAE, Montfavet, France) investigated the impact of phages in agricultural environments, specifically on *Pseudomonas syringae* communities on apricot trees. *Pseudomonas syringae* is a phytopathogenic bacterium that causes significant crop losses. Strains from different phylogroups are involved in bacterial canker disease on apricot trees, with recent research suggesting a closer relation with environmental strains. They isolated and characterized 25 new *P. syringae* phages from apricot tree soils, showing widespread presence and diversity, with 14 genera and 18 new species discovered. Phage infectivity on *P. syringae* strains from different ecological niches (apricot and nonagricultural environments) was tested, identifying two generalist phages belonging to different genera. Moreover, phage infection was found in around 26% of all strains tested. Study of this interaction network revealed a nested structure, with no geographical isolation of *P. syringae* phages, suggesting a continuum of interactions. These results are consistent with the ubiquitous ecology of *P. syringae* (Feltin et al. 2024).

The identification of signals inducing prophages within the gut microbiota is limited. **Caroline Henrot** (PhD student, Institut Pasteur, Paris, and MICALIS, Jouy-en-Josas, France) investigated the potential of bacteriocins produced by *E. coli* as prophage inducers. In the gut, it is estimated that more than half of bacteria carry prophages, and approximately 50% of intestinal *E. coli* have at least one bacteriocin-encoding gene. Moreover, some of these toxins trigger the SOS response in targeted bacteria by activating the regulator RecA, which is also involved in the canonical prophage-induction pathway. A first screening of four strains encoding various bacteriocins and selected among over 900 *E. coli* strains isolated from fecal samples constituting the COPSAC collection led to the identification of two bacteriocin-positive strains capable of inducing prophages. Prophage induction was also observed for various lysogens of the COPSAC collection carrying lambda prophages, but not for others carrying P2 phages. Each of the two positive strains contains one plasmid—carrying, respectively, a colicin with a DNAse domain and a recently identified microcin—that induces a prophage-inducing activity, upon cloning into a laboratory strain. Furthermore, deleting the bacteriocin genes suppressed the induction. Overall, these results suggest that bacterial competition via colicins may shape the gut virome.

As Stephen Jay Gould wrote in *The Flamingo's Smile*: "Variation is the raw material of evolutionary change." Indeed, the generation of random genetic variability occurs via mutations. Hence, estimating the mutation rate in bacteriophages with precision would give fundamental insights into their evolution. **Julien Lopez** (PhD student at the MICALIS Institute, Jouy-en-Josas, France) presented his work on the investigation of the mutation rate in bacteriophage lambda. In fact, in 1968, only one study estimated that the mutation rate of phage lambda is at least 100-fold higher than the one of its host, *E. coli*, based on a mutagenesis study in a single locus (Dove 1968). As phage lambda and its host are both replicated by DNA Pol III, this difference was peculiar. Using precise duplex sequencing technology, having an error rate 10 000-fold lower than classical sequencing methods, Julien Lopez successfully obtained a genome-wide rate of substitution per nucleotide per replication of phage lambda that is six-fold lower than the previous estimation in a single locus. Using a mismatch repair (MMR) system, deficient strain only increased the lambda phage mutation rate by three-fold compared to the wild-type strain, whereas it increased *E. coli* mutational rate by 138-fold; hence, the MMR system is inefficient in terms of determining the mutation rate of the lambda genome. Julien Lopez also explained that the distribution of the mutations was random and uniform across lambda phage genome, suggesting the absence of selection during the sequencing. In the future, he will confirm his DupSeq results and investigate the MMR inefficiency mechanisms on the phage lambda genome.

Different forces will shape the frequencies of the new variants such as genetic drift, gene flow, and natural selection. **Wakinyan Benhamou** (PhD student at the University of Montpellier/Centre d'Ecologie Fonctionnelle et Evolutive, Montpellier, France) presented his work on the statistical inference of life-history traits characterizing new pathogen variants. Berngruber et al. (2013) proposed a mathematical model for the competition between two strains of the temperate phage lambda: a wild-type virus versus a mutant virus, more virulent, with parameters to simulate the temporal dynamics of prevalence (epidemiology) and strain frequencies (evolution). This study notably showed that selection on virulence was time-varying: virulence is selected in an emerging epidemic—when the density of susceptible hosts is high—and then counter-selected at the end of the epidemic—when the pool of susceptible hosts is depleted. However, this theory was not completely fitting the experimental evolution data; especially, simulations failed to properly capture the peak in the dynamics of the virulent phage frequency among infected cells. Wakinyan Benhamou exposed his novel model (Benhamou et al. 2024) to better fit experimental data. He added an additional compartment in the phage life cycle to take into account the lysis time (instantaneous in the previous study). Thanks to this extra step, he improved the goodness of fit with experimental time series. A theoretical analysis of this new model yielded useful insights for the gradient of selection at different stages of the epidemic and the differentiation between host compartments. Finally, he estimated model parameters, and in particular the phenotypic traits of both strains of phage lambda used in this evolution experiment.

The selective pressure can vary across time but can also be of various types. **Coline Meynard-Doumenc** (PhD student at the Centre de Recherche Saint-Antoine, Paris, France) hypothesized that oxidative stress, and more precisely reactive oxygen species (ROS), can modulate the diversity of interactions between bacteria and their phages. To test for that hypothesis, she designed an evolutionary experiment where three *E. coli* strains evolved with one virulent phage in the presence/absence of oxidative stress conditions (hydrogen peroxide) during 10 days in chemostats. In both conditions, phages and bacterial communities co-existed, but the phage-to-bacteria ratios differed, and the diversity of phage host ranges decreased under oxidative stress. Moreover, potential phage–bacteria arm race signatures were assessed through time using genomic analysis. The selective pressure in a controlled synthetic environment, i.e. a proto-ecosystem, allows fundamental understanding of how environmental variations may affect bacteria–phage interactions. Precisely, ROS are critical in the pathogenesis of inflammatory bowel diseases (IBD), characterized by an altered intestinal microbiota. This IBD mimicking synthetic ecosystem could give clues about the undergoing processes in this disease.

In contrast to synthetic ecosystems customized to study independent selective pressures, natural ones are the places for many selective pressures. In each ecosystem, selective pressures act at intraspecific and interspecific scales. **Christina Hazard** presented the work of **Sungeun Lee** (Postdoctoral Fellow at Ecole Centrale de Lyon, Ecully, France) exploring a poorly understood ecosystem: the interactions between phages and their hosts in soil. Understanding of the diversity and impact of viral infection on soil autotrophic bacteria and archaea is lacking, yet potentially critical for such communities regulating key processes such as the global nitrogen cycle and fertilizer losses in soil. To better characterize these interactions, DNA stable isotope probing and metagenomics were used, allowing to track assimilated carbon transfer from prokaryotes to viruses. These methods enabled the identification of novel virus families, notably in ammonia oxidizing archaea (Lee et al. 2023). To detect weaker interactions, virus-like particles from nitrifying soil microcosms were filtered and known characterized interactions were inhibited. This allowed an increase in the abundance of viruses infecting noninhibited groups and the identification of 69 novel virus families in ammonia and nitrite oxidizing bacteria. These results would give insight into host–virus interactions during nitrification. Despite all this, on an understudied ecosystem, **Eric Dugnat-Bony** (Research Scientist at INRAE in the SayFood unit, Palaiseau, France) presented the advances of his team on the interactions between phages and their hosts in cheese rind. Viral communities have been observed coexisting with various microorganisms involved in key steps of the cheese production process such as milk acidification, coagulation, and ripening (Dugat-Bony et al. 2020). To study the unknown ecological relevance of these phages, they isolated five new virulent phages infecting ripening bacteria and characterized them through genome sequencing, electron microscopy, and plaque assays. Then, Polymerase Chain Reaction (PCR) detection was used to check their presence/absence in the cheese factory, revealing equipment and processing surfaces as being the main phage reservoirs. Finally, metagenomics studies indicate that maturation steps were associated with the succession of specific phage communities and correlate with the bacterial dynamics observed on the cheese surface. Some viral genomes were also systematically detected in matured cheese, independent of the production year (Paillet et al. 2024). The characterization of this interactome is promising to optimize cheese production and to understand phage ecology in cheese.

Studying the interactions between phages (bacterial parasites) and their hosts is key toward understanding their co-evolution. But one can also study the interactions between phages and their hyper-parasites. **Jorge Moura De Sousa** (Research Scientist at Institut Pasteur in the Microbial Evolutionary Genomics Team, Paris, France) presented his work on the characterization of phages hijackers: P4-like elements (de Sousa et al. 2023). Phage mobilization is exploited by mobile genetic elements (MGEs) as "phage satellites." These MGEs are not autonomous in transfer as they lack viral particle components, but hijack other "helper phages" particles to be mobilized. P4 satellites are typically described to hijack P2 elements. It has been shown that P4 can be mobilized by other P2-like phages. This is not known for P4-like elements, raising the following question: Can similar satellites hijack the same helpers? Jorge Moura de Sousa showed that P4-like elements are abundant and diverse, as other satellites. Investigating sequences shared by P4 and P2 mechanistically needed for hijacking, he showed that these sequences were also similar between P4- and P2-like phages, with clues also supported experimentally. Altogether, these co-evolutions of hijacker/helper might also help to predict mobilization potential across hosts.

Structure and assembly

Structural biologists, despite representing a rather limited number of teams in the network, showcased recent major methodological developments in the field. Cryo-electron microscopy (Cryo-EM), whether single particle analysis (SPA), as in Paulo Tavares work on SPP1, or tomography, as in Alessio d'Acapito project on T5, now allows us to solve structures at atomic or near-atomic resolution for very large protein complexes. Moreover, AlphaFold2 (AF2) has become an invaluable tool, allowing us to expand past and present structural knowledge to related sequences. This was illustrated by Adeline Goulet in her talk analyzing mycophages (Cambillau and Goulet 2023).

Keynote speaker of the session was **Matthew Dunne** (Research Scientist, ETH Zurich, Switzerland, and Micreos Pharmaceuticals, Switzerland), who shared insights from his research career at ETH Zurich, where he demonstrated how structural data can provide a mechanistic understanding of phage assemblies but also inform the design of phage-based diagnostic and therapeutic applications. The structure of the long tail fiber (LTF) tip of *Salmonella* phage S16 was resolved by X-ray crystallography, revealing a rare polyglycine fold. This fold forms highly variable loops that constitute the binding site to the phage receptor, outer membrane protein C (Dunne et al. 2018). This intriguing architecture closely resembles the complementary determining regions found in antibodies and has been found in the fibers of other phages, including myobacteriophages. Additionally, he presented another mode of receptor binding used by phages, such as the different fiber tips of phages T5 and T4. The S16 LTF was engineered into an enzyme-linked LTF assay, a technology of interest for the food industry due to its ability to provide fast (2 h) results with a low detection limit (10^2 CFU/ml) and high specificity for *Salmonella* (Dunne and Loessner 2019). Furthermore, this expertise in LTF engineering was exploited to develop an agglutination assay for the rapid detection of *Burkholderia pseudomallei*, the causative agent of melioidosis. This assay used an engineered variant of the tail fiber of *Burkholderia* phage E094 (Muangsombut et al. 2021).

Cryo-EM has undergone significant technical development and has allowed many viruses and phage structures to be solved through SPA. **Alessio d'Acapito** (PhD student, Institut de Biologie Structurale, Grenoble, France) presented cryo-electron tomography (Cryo-ET), to observe even larger objects than SPA, in het-

erogeneous environments. High-resolution SPA structural studies on *E. coli* siphophage T5 were carried out on the capsid, the tail tube, and the tail tip and receptor complex (Linares et al. 2023), providing a wealth of information on mechanisms of viral particle assembly, docking to the host surface and DNA delivery to the host cytoplasm. However, the structure and precise nature of the channel forming through the host membranes and periplasm have remained elusive. Using cryo-ET, one can directly observe the infection of phages on *E. coli* minicells, and higher resolution structural information can be obtained by sub-tomogram averaging. Alessio's PhD project is to adapt such a method to visualize *in vivo* the remodeling of T5 and its host cell wall occurring upon infection initiation.

The DNA of tailed phages is translocated into a preassembled procapsid in a reaction energized by ATPase hydrolysis. This translocation is carried out by a motor assembled at the dodecameric portal found at a single vertex of the procapsid. **Paulo Tavares** (Research Scientist, Institute for Integrative Biology of the Cell, Gif-sur-Yvette, France) presented a 2.7 Å Cryo-EM structure of the *Bacillus* phage SPP1 portal protein (Gp6) in complex with head completion proteins Gp15 and Gp16. The $gp16_{12}gp15_{12}gp16_6$ complex, called connector, forms after disassembly of the DNA motor to prevent leakage of the packaged DNA and allows binding of the tail to form mature phage particles (Orlov et al. 2022). This structure was compared with previously acquired structural data and biochemical studies of different mutants to propose a molecular mechanism for sequential polymerization of gp15 and gp16 at the portal vertex. Furthermore, secondary structure elements involved in "gatekeeping" were identified: most notably gp6 tunnel loop $\alpha5'$-$\alpha6$, acting as a ratchet preventing DNA sliding out during packaging, and gp16 loops $\beta2'$-$\beta3$ folding as helices blocking the tunnel's lumen and insuring DNA retention. Moreover, architectures of portal and head-to-tail connectors can be used to map the evolution of the lineage of tailed-prokaryotic and herpes viruses.

Although there are over 12 000 identified mycobacteriophages infecting different mycobacteria, the way in which these mycobacteriophages bind to their host envelope remains poorly understood. **Adeline Goulet** (Research Scientist, Laboratoire d'ingénierie des systèmes macromoléculaires, Marseille, France) uses AF2 to predict the structure of the proteins assembling the mycobacteriophage host-binding machineries and get insights into their host-binding mechanisms. These predictions highlight unique structural features for these modular machineries. They contain a receptor-binding protein that, for most of the studied mycobacteriophages, comes along with two additional proteins also likely involved in host binding. These proteins include carbohydrate-binding modules and lectin-like domains, likely involved in the binding of polysaccharides, as well as unusual polyglycine-rich domains (PGDs). In the *Salmonella* phage S16, the PGD located at its tail fiber adhesin is involved in the specific recognition and binding of its host. This AF2-based approach offers the possibility to explore structure–function relationships of phage host-binding machineries and thus provides valuable information to design tools for the detection and treatment of bacterial infections.

Phage and host interaction

In order to initiate an infectious cycle, a viral particle must make its way to the surface of a target where one or several features will be specifically recognized. **Stefanie Barbirz** (Research Scientist, Medical School Berlin, Berlin, Germany) presented studies and methods developed in her lab focused on *Salmonella* infecting phages, their behavior at the host surface, and surroundings that condition infectivity. Polysaccharides are a major constituent of biofilms, extracellular matrices, and condition access to the cell surface. A method was developed to measure the effect of increasing concentrations of exopolysaccharides, purified from *Pantoea stewartii*, on nanoparticles and phage particle diffusion. Another barrier defence mechanism phages have to overcome to reach their host cells is the shedding of outer membrane vesicles. When under stress, notably during phage infection, these vesicles carrying lipids, proteins, and glycan of the outer membrane are susceptible to reducing amounts of infective particles by triggering phage DNA release in solution. This phenomenon was characterized *in vitro* with phage P22 (Stephan et al. 2020). To study phage behavior at the cell surface, *in vitro* systems are worked on, such as giant unilamellar vesicles (Stephan et al. 2023) and supported lipid bilayer. Finally, the phage DNA release of lipopolysaccharide binding phages can also be studied *in vitro*, with kinetic parameters measured and great variation between different phages established.

Viral communities, called virome, have potential as disease biomarkers within a microbiota context, but still remain poorly studied. **Quentin Lamy-Besnier** (Postdoctorate, MICALIS, Jouy-en-Josas and Centre de Recherche Saint-Antoine, Paris, France) studied the virome of patients with Crohn's disease (CD) in blood and feces samples. He developed methods to overcome the specific limitations of those environments (low quantity of viruses, contaminants), and reported in their first results the identification of hundreds of phages in the human blood. They demonstrated that the contigs present in the blood virome are rarely found in the intestinal virome. They revealed significant differences in the blood virome composition between CD and healthy patients (Lamy-Besnier et al. 2024). However, no significant difference was observed in the fecal virome between the two groups.

CD is associated with alterations in gut barrier function, notably a hyperpermeability, and in the microbiota, largely composed of bacteria and phages. **Clara Douadi** (Postdoctorate, Centre de Recherche Saint-Antoine, Paris, France) showed that the phage contigs found in both blood and stools are in a significantly higher amount in CD patients compared to healthy individuals, suggesting that the increased gut intestinal permeability in CD patients could facilitate phage translocation. Therefore, she studied in depth the translocation of phages from the intestinal lumen to the blood with *in vitro* and *ex vivo* approaches. The phages T4, M13, and ϕX174 are able to internalize and cross the intestinal epithelial cells without inducing inflammation, cytotoxicity, or hyperpermeability, with an increase of ϕX174 phage translocation when intercellular junctions are destabilized. They also showed using fluorescent phages that these viruses can internalize endothelial cells, suggesting that they would be able to reach the bloodstream. They also confirmed that phages translocated across mouse intestinal tissues. These results strengthen the hypothesis that phages can cross the intestinal barrier to reach the bloodstream in a phage-dependent manner when the intestinal barrier is altered, and allow a better understanding of phage dynamics in IBD.

Polymorphic toxins (PTs) are a large family of toxins involved in interbacterial competition and pathogenesis. These modular proteins are composed of a conserved N-terminal domain responsible for transport and a variable C-terminal domain carrying toxic activity. Bioinformatics analyses have identified a new family of putative PTs containing an MuF N-terminal domain in the genetic elements of the prophage resembling the F protein of col-

iphage Mu. **Julie Viala** (Research Scientist, Laboratoire d'Ingénierie des Systèmes Macromoléculaires, Marseille, France) has characterized the MuF-RelA_SpoT toxin associated with the temperate *Streptococcus pneumoniae* SPNA45 phage. Her work shows that the RelA_SpoT domain (C-ter) possesses (p)ppApp synthetase activity, which is bactericidal under their experimental conditions. She also highlighted the two downstream genes encoding two immune proteins, one binding and inactivating the toxin and the other detoxifying the cell via (p)ppApp hydrolase activity. She proposes a signature to more easily distinguish (p)ppApp synthetases from (p)ppGpp synthetases on the basis of protein sequence alignments (Bartoli et al. 2023).

Neisseria meningitidis, a human nasopharyngeal bacterium, can cause serious illness. The MDA filamentous phage (meningococcal disease associated) has been identified as linked to invasive meningococcal disease, increasing bacterial colonization and promoting infection. **Clémence Mouville** (PhD student, Institut Necker Enfants-Malades, Paris, France) shows with deletion mutants that MDA phage entry requires a functional and retractable type IV pili (TFP). The main pilin (PilE) forms the TFP fiber and the MDA bundle together. PilE variants expressing more positive charges were associated with MDA entry, suggesting a direct interaction. Amino acid analysis confirms this hypothesis. The study also points out that T4P variants whose electrostatic potential favors phage infection allow stronger adhesion of bacteria to endothelial cells than variants less infected by phages. These results support a model of interaction between filamentous phages and type IV pili favoring colonization of the endothelium by infected bacteria, and thus indirectly participating in the selection of pathogenic strains.

Filamentous phages are nonlytic viruses that form persistent associations with their host. They can have a significant impact on the physiology of the bacterial host or introduce new pathogenic factors. Although studies on coliphages M13, fd, and f1 have shed light on certain aspects of the *E. coli* infection process, the mechanisms underlying periplasmic translocation and insertion into the inner membrane remain poorly understood. **Callypso Pellegri** (PhD student, Laboratoire d'Ingénierie des Systèmes Macromoléculaires, Marseille, France) recently identified new host partners involved in phage translocation. *In vivo* studies using biochemical techniques revealed an interaction between the phage spike adhesion protein pIII and TolQ and TolR, two inner membrane proteins forming the proton-dependent molecular motor of the Tol–Pal system. These interactions occur between the transmembrane helical domains of pIII, TolQ, and TolR, potentially responding to the proton motive force in the cell. A new model for the late phase of filamentous phage translocation is proposed, involving multiple interactions with each component of the TolQRA host complex (Pellegri et al. 2023).

Amel Chaïb (Postdoctoral Fellow, ISVV, Bordeaux, France) presented observations made during a protocol aimed at isolating bacterial insensitive mutants (BIMs) to phages in *Oenococcus oeni*, which performs malolactic fermentation of wines. BIM characterization showed phenotypic modifications affecting growth, phage sensitivity, or colony morphology. However, several subculturing phages persisted, indicating the presence of still-sensitive bacteria in cultures, independently of the virulent phage challenge. Further analyses showed the presence of sensitive bacteria, carrying phage or not, alongside BIMs. Moreover, phage-carrying sensitive individuals were able to generate BIMs, and uninfected and infected bacteria again. These results suggest a carrier state life cycle where sensitive and resistant bacteria, along with phages, persist at the population level. Such cohabitation may promote bacterial fitness as described in other models, in addition to enabling virulent phages to remain protected from harsh wine conditions within their better-adapted bacterial host.

In fact, the evolution of bacteria to become resistant to phages can lead to a decrease in bacterial fitness, as demonstrated also by **Anaelle BAUD** (PhD student, Laboratoire d'Ecologie Microbienne, Lyon, France). Indeed, following the characterization of several lytic phages targeting the lettuce pathogen *Xanthomonas hortorum* pv. *vitians* (Xhv), analyses identified the O-antigen, present in the bacterial cell wall of Gram-negative bacteria, as the primary receptor for phage Xhv1. Intriguingly, mutant bacteria that became resistant to this phage exhibited reduced fitness in plants and impaired mobility in soft agar assays, creating a significant trade-off in plants. These findings support the design of a phage cocktail to prevent resistance, thereby ensuring the sustainability of the plant.

From predation with cell lysis to mutualistic benefits through lysogenic conversion, the relationships between phages and their hosts are complex. Coevolution has led to the emergence of specialized antiphage systems in bacteria, and phages evolved to evade bacterial defenses. Bacteria developed the general stress response system, regulated by the sigma factor RpoS. To prevent the general stress response from interfering with their viral cycle, some phage species have evolved proteins that interact directly with RpoS, such as the T7 Gp5.7 protein (preventing host RNA polymerase from functioning) or T4 AsiA protein (redirecting host RNAP toward viral gene transcription). **Nicolas Ginet** (Research Scientist, Laboratoire de Chimie Bactérienne, Marseille, France) has discovered the T5 Gp00X protein, a 31 amino acid viral pre-early protein transcribed and translated during host takeover. In *E. coli*, he shows that Gp00X induces RpoS proteolysis by the bacterial ClpXP protease, independently of the adaptor RssB. This prevents the establishment of a general stress response, favoring the use of sigma70 rather than RpoS for transcription of viral genes by the host RNA polymerase. This discovery reveals a new phage strategy for the regulation of RpoS in *E. coli*.

Clarisse Plantady (PhD student, Institut Cochin, Paris and Phaxiam Therapeutics, Lyon, France) explored the main causes of inherent phage resistance in clinical strains of *Pseudomonas aeruginosa*. She used nine anti-*P. aeruginosa* phages against a panel of 125 clinical strains associated with antibiotic treatment failure. In this interaction matrix, 73% of the strains exhibited susceptibility to at least one phage. For the resistant strains, the first step was to determine whether the resistance simply resulted from a lack of phage receptor and the preliminary results indicated that phage adsorption is not always impaired. Subsequently, the bacterial genomes were analyzed to determine the possible involvement of intracellular defense systems. Using DefenseFinder 83 subtypes of antiphage systems were identified. This study will provide insights into whether bacteria generally need to carry multiple defense systems or specific combinations to resist different types of phages. Such an understanding is crucial for the design of phage cocktails to mitigate the impact of phage resistance on therapeutic outcomes.

Clostridium difficile is an attractive target for phage therapy as it is a common nosocomial pathogen taking advantage of the dysbiosis of the gut flora upon use of broad-spectrum antibiotics. *Clostridium difficile* cells are coated with a surface layer (S-layer), a proteinaceous two-dimensional *para*-crystalline array. **Alexia Royer** (PhD student, Institute for Integrative Biology of the Cell, France, and University of Sherbrooke, Canada) presented her study in which S-Layer Protein A (*slpA*), a major component of the surface layer, was identified as a receptor for many sipho- and

myo-phages (Royer et al. 2023a). Indeed, *C. difficile* strain FM2.5, lacking *slpA*, exhibits resistance to eight phages. Adsorption was also shown to be impaired on the FM2.5 strain. Complementation strains using 12 different isoforms of *slpA* were tested against eight phages (three siphophages and five myophages), showing restored sensitivity to phage infection depending on the isoform type as well as phage tail architecture. A domain, called D2, on the nonconserved low molecular fragment of *slpA*, was determined to be important for infection by some phages.

Hemolytic uremic syndrome is caused by shiga toxin-producing *E. coli*; however nonpathogenic *E. coli* strains infected with 933W temperate phage (which carries genes of the two shiga toxin subunits) can also cause this syndrome (Del Cogliano et al. 2018). **Marie-Agnès Petit** (Research Scientist, Micalis Institute, Jouy-en-Josas, France), reported data of mice colonized by nonpathogenic *E. coli* lysogenized with phage 933W. Damages caused to different organs as well as presence of phage particles were assessed. Surprisingly, despite cells being inoculated intragastrically, phage particles were found in the central nervous system (corpus callosum and hypothalamus). Moreover, although invasion levels remained the same throughout the mouse bodies, upon deletion of pr1, a eukaryotic promoter upstream of the toxin gene *stxA2* in the prophage sequence, the amount of phage particles observed and tissue damages in the brain were substantially reduced.

Therapy and biotechnology

The fourth session of this congress included the potential applications of phages, not only in the medical domain but also in the industrial sector. **Ran Nir-Paz** (Clinician, Hadassah Medical Center in Israel) introduced this session by presenting advancements in the field of phage therapy in Israel. The collaboration between Hadassah Medical Center and the Hebrew University has led to the establishment of the Israeli Phage Therapy Center (IPTC). This center has created a bank of over 500 phages along with accompanying structures and services. It has created an extensive pipeline for clinical phage microbiology, facilitating a more extensive use of phages as a treatment in Israel and globally, with a success rate ranging from 70% to 86%. Furthermore, the center systematically collects and normalizes data from treated patients to optimize the use of phages in therapeutic interventions (Yerushalmy et al. 2020, Yerushalmy et al. 2023, Onallah et al. 2023).

The research aimed at advancing phage therapy is intensifying, particularly in exploring various means of administering these phages to target infection sites. A concrete example is the oral administration of phages for decolonizing the gut of pathogenic strains that can cause extraintestinal infections, as presented by **Floriane Laumay** (MCU, Centre International de Recherche en Infectiologie, Lyon, France). In her work carried out in collaboration with the national centers for antibiotic resistance of Clermont-Ferrand and Bicêtre university hospitals, a single oral dose of a combination of a *Tequatrovirus* phage and a *Vectrevirus* phage showing complementary host spectra triggered a complete decolonization of the mice intestine of a carbapenem-resistant *E. coli* strain belonging to the sequence type 131, a major lineage of *E. coli* responsible for frequent urinary infections in humans. In-depth investigations revealed an increased adsorption of the *Tequatrovirus* phage on strains becoming resistant to the *Vectrevirus* phage both *in vitro* and *in vivo*, suggesting a potential synergistic effect between these two phages.

Cindy Fevre (Chief Scientific Officer of Phaxiam Therapeutics, Lyon, France) presented the preclinical development of phage therapy against *P. aeruginosa*, which is the main cause of nosocomial lung infections. A combination of four phages has been developed for specific administration by nebulization. This presentation focused on the difficulty to define the dose, which is fundamental to ensure therapeutic success, and which is one of the most important rationales reviewed by health authorities such as the European Medicine Agency (EMA) and the Food and Drug Administration (FDA). The difficulty relies on the fact that the phage active dose varies from one strain to another, and that the replicative nature of the phage complicates the pharmacokinetic study design. The host range of the four phages was presented (88%) with an emphasis on the necessity to add an assay to determine the minimum active dose (Le Guellec et al. 2023). They focused on the deliverable dose of this phage combination using *in vitro* models mimicking the mechanical ventilation system, and performing exposure studies on nonhuman primates. Results show that 5%–10% of the phages loaded in the nebulizer reached the lungs in *in vitro* and *in vivo* models. Although low, this survival rate of the phage is enough to reach the minimal active dose determined *in vitro*. The four-phage combination was also shown to be active at the defined dose *in vivo* models of lung infections (Guillon et al. 2021). Toxicology studies were also conducted to ensure the safety of therapeutic use of phages. Administration of 1×10^{10} PFU/kg/day for dogs and 1.43×10^{9} PFU/kg/day for rats for 14 days was well tolerated locally. Altogether, these results constitute the backbone of the preclinical data included in the regulatory dossier to obtain the authorization to conduct randomized double-blinded clinical trials that are necessary to get phage therapy approved and widely integrated into the antimicrobial therapeutic arsenal.

For now, phages have an undefined regulatory network in Western Europe, and phage therapy applications are relatively scattered. For now, Belgium is pioneering the use of personalized phage therapy by featuring the most established and innovative regulation, with a new, pragmatic regulatory framework, guided by a monograph since 2018. Phages are considered in Belgium "active pharmaceutical ingredients" and can be prepared and combined according to this monograph. The latter states that the phages must be prepared at the Queen Astrid Military Hospital and their quality control ensured by Sciensano. **Mathieu De Jode** (Postdoctorate, Sciensano, Ixells, Belgium) presented the quality control process of clinical phage products: the quality control starts with the construction of a genomic passport to ensure that the phage is strictly lytic and does not contain any AMR or virulence gene. Then, each clinical phage lot is titrated and checked for identity, and microbial and prophage contaminants. To date, the quality control process has ensured the safety of more than 50 distinct phage batches used in the treatment of over 100 patients (Pirnay et al. 2024).

Despite promising results, phage therapy faces multiple challenges. For instance, **Camille Kolenda** (Research Scientist, Hospices Civils de Lyon, Université de Lyon, France) stated that the therapeutic potential of anti-*S. aureus* is insufficiently characterized, and bacterial determinants of phage activity, such as phage receptors and antiphage systems, are not explored enough as well. She presented her work on the characterization of the therapeutic potential of anti-*S. aureus* phages. She evaluated the activity of a panel of anti-*S. aureus* phages, where findings suggest a host complementarity. She presented her *in silico* approach to identify putative bacterial determinants of phage activity and determined that there is only a little impact of the wall teichoic acid on phage activity. She could also identify a diversity of putative antiphages systems. This in-depth *in vitro* characterization of phages exhibiting high therapeutic potential and complementarity against *Staphy-*

Figure 1. Picture of the participants of the eighth annual meeting of the Réseau Bactériophage France.

Figure 2. Picture of the contest "Build your Phage" with Lego blocks.

lococcus species, as well as the *in-silico* study, provides novel perspectives for phage susceptibility evaluation and identification of phage activity determinants.

To further characterize phages and their therapeutic potential, multiple biotechnologies are being developed to overcome the limitations of culture-based methods. The aim of SUPPLY (superfast photonic detection of phage lysis) is to develop a device based on biophotonic microsystems to comprehensively study phage–bacteria interactions, which currently rely solely on direct visual detection of plaques. **Pierre R. Marcoux** (Research Engineer, Université Grenoble Alpes, CEA, Grenoble, France) presented advancements in optical trapping and nondestructive characterization at the single-cell scale of bacteria being trapped inside lineic microcavities or bidimensional photonic crystals (Villa et al. 2024). The study explored the optical characterization of the membrane modifications of the trapped cell in various stresses such as thermal, osmotic, antibiotic, and lytic phage stresses. Findings suggest that on-chip devices for optical trapping may allow a significant enhancement in bacteria characterization at the single-cell scale, facilitating rapid antimicrobial susceptibility testing.

Furthermore, a key aspect of phage therapy lies in the research of strictly lytic phages with a broad host spectrum. However, this pursuit can pose a challenge for certain pathogens, such as *C. difficile*, whose phages are inherently nonstrictly lytic and exhibit a narrow host spectrum. Fortunately, promising solutions exist, as exemplified by phage engineering presented by **Louis-Charles Fortier** (Research Scientist, Université de Sherbrooke, Sherbrooke, Canada). Indeed, the mutation of RBPs (Royer et al. 2023b) and the deletion of genes corresponding to global repressors and integrase of temperate phages enable the generation of strictly lytic phages, tailored to the desired host spectrum through DNA modification using CRISPR-Cas. This approach thus facilitates the personalized design of phages for optimal patient treatment.

Furthermore, the study of phages is gaining momentum, not only in the field of phage therapy but also in other contexts such as prevention. Indeed, **Pascale Boulanger** (Research Scientist, Insti-

tute of Integrative Biology of the Cell, Gif-sur-Yvette, France) presented the use of nanoparticles derived from phages as novel vaccine vectors by attaching antigens to the capsid of phage T5. This capsid-like particle is nonreplicative as it lacks phage DNA and can affix the antigen by genetic fusion with a decoration protein, pb10, possessing a strong affinity for the T5 capsid. Such vaccines have notably triggered a robust and durable immune response against the model antigen ovalbumin in mice, thus holding potential medical interest for the development of a versatile vaccine platform that is easy to produce, cost-effective, and highly stable (Vernhes et al. 2024).

Conclusions

With a total of 163 participants (Fig. 1), 92 women and 71 men, among which about 5% came from outside France, the meeting has confirmed its strong value for the French community while also drawing the attention of scientific communities from abroad. During the gala dinner, a contest was organized between different tables to build their own phage using Lego (Fig. 2). The participants, who largely appreciated this entertainment, are now waiting for the next one that will be proposed by the organizers of the ninth meeting, which will take place near Sète in November 2024. Moreover, the network of phage teams has decided to launch a webinar series to be held monthly every last Wednesday for Early Career researchers who will present their ongoing projects (www.phages.fr). Last but not least, a new working group, "ApprentiPhages.fr," was initiated to share know-how and materials to foster teaching courses using phages.

Acknowledgments

The eight meeting of French Phage Network (phages in Lyon) was supported by CNRS, INRAE, INSERM, SPILF, SFM, ESGS-ESCMID, LESAFFRE, CIRI, PHAXIAM, and the Hospices Civils de Lyon. P.-A.P. is supported by the Fondation pour la Recherche Médicale.

Supplementary data

Supplementary data is available at *FEMSML Journal* online.

Conflict of interest: None declared.

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Received 11 November 2024; **revised** 2 January 2025; **accepted** 16 January 2025