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## Thinking phage innovations through evolution and ecology

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### Authorship statement:

Charlotte Brives: conceptualization, writing of original draft, review and editing; Rémy Froissart: writing of original draft; Blanca Perez-Sepulveda: writing of original draft; Claire Le Marrec: writing of original draft.

### Abstract:

In this article, we conduct an interdisciplinary review of the potential of phage-based applications in light of current knowledge about phage evolution and ecology. Gaining an improved understanding of phages' ecology and evolutionary dynamics is crucial for recognising both the benefits and limits of their usage, as well as potential negative downstream effects across different ecological milieus. As a reference, the history of the industrialisation of antibiotics and the rise of antimicrobial resistance act as a reminder of the deep entanglement of both the evolvability capacities of micro-organisms and the history of human societies. Based on evolutionary biological parameters, we show that (i) virulent bacteriophages are best candidates for biocontrol, (ii) best cocktails harbour complementary bacteriophages preventing bacterial cross-resistance and (iii) cure can also be considered with steer of bacterial bacteriophage-resistance evolution toward loss of virulence factor and/or increase in antibiotic susceptibility. A detailed review of what is known about the role of phages in vine cultivation and wine production finally serves as an example to show how it is important to consider site-specific rather than one-size-fits-all responses.

## Introduction

The last few decades have seen unprecedented recognition of the role of microorganisms in many ecosystems, from the human gut to biogeochemical cycles in forests and oceans (1, 2). The usual dichotomies that humans use to think about microbes (and about other living organisms more generally), between pathogens and beneficials, friends and enemies, individuals and communities, are beginning to break down (3, 4, 5, 6). Microorganisms are everywhere, with roles and functions that are highly variable and milieu-dependent, situated in time and space.

Study of microbes has led to profound conceptual changes both in the production of knowledge in biology (7) and in their possible uses to benefit humans, whether in health, agriculture, or industry. They are now viewed as essential to the development and maintenance of macro-organisms, maintaining symbiotic and co-dependent relationships with them. These macro-organisms are, at least for a part of the life sciences community, no longer considered as individual entities but as multi-specific and symbiotic complexes (8) with an “extended phenotype” (9).

This redefinition of ecosystems, organisms, and their components is accompanied by a reconsideration of ecologies, states of health, and the aetiologies of many pathologies, which are now considered from the perspective of eubiotic ecological equilibrium (*i.e.* microbiota associated to healthy state) or of dysbiosis. Often enough, this redefinition has taken place even if the actual mechanisms at work have not yet been elucidated. Microbes have thus become mediators that can be used to develop responses to problems encountered by humans, whether scientific, technical, ecological, or medical.

Faced with the major challenges generated by human activities, such as pollution, global warming, or the general reduction of biodiversity, or antimicrobial resistance (AMR), bacteriophages (phages), due to their specific and rich relationships with bacteria, appear to hold great promise. However, these promises are highly dependent on the way in which phages are thought of, used, and applied in these different sectors, and on the way in which this is articulated with the existing knowledge (or lack of), contexts and infrastructures. Phages have been used for over a hundred years in molecular biology, typing, or human health with varying degrees of success. In human and animal health, agriculture or biocontrol, however, the frameworks of usage are still experimental. In human health for example, phages have to fit with evolving regulations and techniques but also with the ever-evolving state-of-knowledge. As the shape of phage potentialities for human, animal, and environmental care are not yet crystallized, as the various standards and protocols are still in the making, this is a very fascinating and exciting moment: an opportunity to think collectively about what it means to think and deal with phage evolvability and phage ecologies.

More than ever, therefore, we need to encourage open discussions between the different disciplines constituting life and social sciences, from microbiology to anthropology to support, accompany, and question the use of phages potentialities.

In this review, we weave together lines of thought from across humanities and life sciences in an attempt to highlight what an interdisciplinary analysis of phage evolution and ecology can contribute to thinking about biotechnological innovations in a context of ecological crisis. We argue that findings from phage ecology and evolutionary dynamics cast light on the benefits and limitations of bacteriophage applications in the fields of antibacterial therapy, biocontrol, and biotechnology, highlighting the dangers of unintended downstream consequences. Writing for an interdisciplinary audience, we believe it is worth explaining certain facts that are well known to biologists, and that will enable researchers in the humanities and social sciences to gain a better understanding of the issues and particularities associated with the use of phages outside the laboratory.

## 1 - Evolution and microgeohistories

Phages replicate through two main types of biological cycle: lytic and lysogenic. Virulent phages replicate through lytic cycle that involves adsorption of the phage particle onto the bacterium, insertion of its genetic material and hijacking of the host's cellular machinery to replicate. The bacterial membrane is lysed after replication and new phages are released, leading to potential infection of surrounding susceptible bacteria. The lysogenic cycle involves the adsorption and integration of the temperate phage genetic material into the bacterial genome. The integrated phage genome is then called a "prophage" and replicates along the bacterial chromosome (vertical transmission) instead of immediately lysing the host, and lysogenic bacteria then become "immune" to further infection by the same type of bacteriophages (a phenomenon called "exclusion to superinfection"). Excision of the prophage can be induced by environmental stresses or DNA-damaging chemicals, such as antibiotics, and enter a lytic cycle. Notably, when prophages excise from the bacterial chromosome, they end up appropriating pieces of bacterial DNA, sometimes leading to horizontal transfers of antibiotic-resistant genes (HGT) between bacteria (10, 11, 12). Temperate phages' propensity for exclusion to superinfection, HGT and for being able to engage a long-term beneficial interaction with their host (13, 14, 15) are some of the arguments for avoiding them in phage therapy. Therefore, only virulent phages or temperate phages engineered into virulent ones (16) are used in phage therapy as they quickly kill the host directly after infection. Knowledge of the mechanisms of phage/bacteria interaction and co-evolution is therefore essential if we are to make good use of their skills.

Successful phage applications - either in biocontrol or for technology development - on pathogenic bacteria depend on knowledge of the precise molecular mechanisms leading to host range and bacterial resistance. Biocontrol aims at reducing and inhibiting all bacterial growth, i.e. preventing any selection of a new emerging bacterial genotype resistant to therapeutic treatment. This goal could be achieved either by using a single "generalist" bacteriophage or a bacteriophage cocktail effective against all potential bacterial genotypes.

Host range can be defined as a qualitative measure: a bacteriophage does or does not infect a collection of bacterial isolates. Success or failure of the bacterial infection depends on the completeness of several steps: adsorption to the bacterial cell surface molecules (phage receptors), injection, expression, & replication of the viral genetic material within the host cytoplasm, and finally the lysis of the cell. Bacteriophage adaptation to a bacterial host thus requires a complex series of matches and adaptations, which may explain why most bacteriophage species target only a few bacterial strains (17, 18) versus others that can target larger host range (19). Bacteria can evolve by acquiring numerous mutations that can act as counter-measures to resist phage infection, which lead to a further specificity-inducing cycle of resistance and counter-resistance (20, 21). Interestingly, scientists might exploit the evolutionary ability of bacteria facing bacteriophages to the benefits of health control (see below). Ultimately, success or failure of a phage infection thus depends on multiple factors (biological and/or environmental) such as presence/absence/level of regulation of mechanisms of bacterial resistance (22) and phages' ability to evade host defences and exploit their host (23).

Each phage-bacteria pairing can thus be regarded as a unique "microgeohistory": the cumulative result of situated encounters, in time and space, of phage and bacteria, leading to unexpected and new evolving pathways (24). The resulting specificity has enabled phages to be used as technical tools to differentiate between, study, and monitor pathogenic bacterial species (see article 3 of the present SI) or as an alternative and/or a complement to antibiotic therapy to combat bacterial infection. Chemical antibiotics usually target bacterial structures such as cell wall or membrane integrity, or essential molecular mechanisms like DNA/RNA, protein, or folate synthesis, making them capable of targeting a wide range of bacteria (e.g. Polymyxin targets most Gram negative bacteria, Vancomycin targets most Gram positive bacteria; and the Carbapenems target both Gram positive and negative bacteria). The use of chemical antibiotics can lead to negative side effects due to the death of other non-pathogenic (commensal) bacteria. Phages' specificity enables a more targeted form of intervention by leaving non-targeted commensal and phylogenetically distant bacteria (e.g. pathogenic enterobacteria *versus* commensal lactobacilli) unharmed. Taking the above-mentioned concept of specificity as a manifestation of phage-bacterial microgeohistories also implies that it is possible to find phages targeting a bacterium in the precise ecosystem from which this bacterium has emerged (25).

Phages, as viruses, are so-called 'obligate parasites', which means that they cannot replicate without their bacterial host. For some virologists and philosophers of biology, viruses' dependence on their hosts makes it inappropriate to classify them in the living world: unable to survive on their own, they are 'on the fringe'; not living, but not inorganic. Others have proposed new conceptualizations which permit the incorporation of viruses into the living realm (26), like Forterre's "Virocell" (27). But it is possible to think of the problem differently: a strict parasite cannot be thought of without its host insofar as its existence is only made possible by the relationship it establishes with that precise host. What viruses force us to think about, then, is less the boundary between the living and the inert than the fundamentally relational nature of biological entities. When we examine the complex

relationships between phages and bacteria, we quickly abandon the narratives that make phages into simple 'parasites', 'snipers' or 'professional killers'. The very relationship of phages and bacteria cannot be so simply summed up as parasitism, especially when we look at the lysogenic cycle. Rather, what we have are spectrums of relationships, with degrees of parasitism, mutualism, symbiosis, each of which may, at some point, and always transiently, characterize the relationship between phages and bacteria (28, 29, 30). Moreover, the study of these relationships reveals more than a spectrum of relational modalities: it brings into play the very plasticity of the entities, their common future in and through the relationships they establish. Phages and bacteria mutually transform each other, according to various modalities and mechanisms, some of which we will return on below. The observation of viruses and bacteria thus offers us the possibility of apprehending "pluribiosis", i.e. spectrums of plural relations between entities that are always in the process of becoming, worked on and transformed by their encounters with other living beings (31). Entities, relations and environments are fundamentally fluid and profoundly relational. Phage/bacteria interactions define never-ending microgeohistories, enabling scientists to develop for instance phage-typing, phage therapy, but also numerous molecular tools, the last one being CRISPR-Cas9 (32).

As we will see below, pluribiotic conceptualisation of phage-bacteria relations has significant implications for the use of phages as therapeutics

In contrast to the significant body of scientific research that has been devoted to studying the evolution of bacterial resistance against chemical antibiotics, much less attention has focused on the mechanisms underlying phage-host adaptation. Until recently, very little was known about the genetic basis of host determinants involved in resistance mechanisms associated either with cell surface-exposed molecules or downstream infectivity pathways.

Different bacterial molecules have been identified to be targeted by bacteriophages as receptors (33, 34, 35). Interestingly, phages can encode other functions involved in central bacterial metabolism, amino acid biosynthesis, translation, regulation of RNA expressions, ion and peptide transports, signalling, and other proteins with less clear functions (36, 37, 38, 39). The described results highlight why some resistance mechanisms may induce differential bacterial fitness selective costs that will drive (or not) increase in frequencies of bacteriophage-resistant bacteria at the within-host and within-environment levels (40, 41).

While researchers are exploring how to mitigate the evolution of bacterial resistance to therapeutic phages (42, 43), bacteriophage specificity (i.e. narrow host-range) could also represent a weakness because of its inefficacy to control other genotypes closely phylogenetically related to the etiologic bacteria. The origin of those genotypes resisting the therapeutic bacteriophage (thanks to slight variation in receptor or non-receptor host factors) can be related to two processes : they might be (i) already present in the infectious site (standing genetic variation) and take over the ecological niche left by the originally targeted pathogen or (ii) coming from the natural selection of a mutant of the targeted etiologic agent. In order to circumvent potential weakness induced by too narrow host-range, scientists proposed to design cocktails composed with bacteriophages for which bacteria use different molecular mechanisms to resist to. So, becoming resistant to one bacteriophage does not lead

to cross-resistance to other bacteriophages present in the cocktail (35, 36). Scientists also suggest to take advantage of bacteriophage evolvability and proposed to both expand host-range and increase virulence of therapeutic bacteriophages. This involves using technical protocols developed a century ago and experimentally evolving bacteriophages through serial passages on a bacterial population composed by multiple diverse bacterial host genotypes. Following the so-called “Appelmans’ protocol” (44, 45), bacteriophages have then been shown to adapt quickly to multiple hosts (mostly different genotypes of the same species), thanks to point mutations allowing host-range expansion (46) and/or to recombination when several related bacteriophage species are co-inoculated during the serial passages (47, 48, 49). Interestingly, such experimental adaptation of bacteriophages also allows to minimize bacterial evolution toward bacteriophage-resistance because adapted bacteriophages maintain bacterial population at low sizes, thus reducing the probability of emergence of new resistant genotypes (50).

Scientists also suggest to take advantage of bacterial evolvability when bacteria face bacteriophage selection pressure (a process called “bacteriophage steering”). In fact, some bacteriophages use receptors that are the cause of disease and physiological disorders. Therapy may then consist in introducing bacteriophages in the infected site to “direct” bacterial evolution toward bacteriophage-resistance by receptor removal. On the one hand, some bacteriophages use flagella (e.g. *Vibrio cholerae*) or molecules (LPS or pilus) outside the cells (e.g. *Yersinia pestis* or *E. coli*) as receptors, which also represent virulent factors that induce bacterial host disorders (e.g. inflammation, colonization, endocarditis, meningitis, sepsis, etc). The only bacterial genotypes able to survive in the presence of therapeutic bacteriophages are then the ones that do not express the specific receptors and consequently become avirulent (51, 52). On the other hand, some bacteriophages use bacterial protein involved in chemical antibiotic resistance such as efflux pump. Following the same evolutionary process, once therapeutic bacteriophage targeting these proteins are applied, natural selection pressure will favorise bacterial genotypes that do not express efflux pumps, thus becoming sensitive to chemical antibiotic (53, 54, 55).

Such experiments underline that innovation in phage therapy can be envisaged not only through phage biotechnology, engineering (56) and synthetic biology (57), but also through the natural selection of (i) evolved bacteriophages *in vitro* to increase their host range and virulence against one or more genotypes of aetiological bacteria chosen locally by practitioners and (ii) bacteriophage-resistant bacteria that become either avirulent or sensitive to chemical antibiotics.

## 2- ecology as entangled multispecies histories

However, while phages can be used in a number of ways, we shouldn’t forget the different roles they can take in different settings. In that sense, pluribiosis is also a practical term to say in one word that neither relations, nor the entities that interact, can be considered fixed once and for all. Which means that humans cannot predict with absolute certainty what will result from the use of microorganisms in their projects, be they about human health, animal health,

or biocontrol. For this very reason, using microbes in human projects is neither without risk nor without consequences. The history of industrialisation of antibiotic production is a paradigmatic example. First, it is a warning. In a paper on the rise of AMR, historian of science Hannah Landecker notes that bacteria bear in their own genomes the history of human societies (58). Hannah Landecker uses the expression “biology of history” to explain how the massive and often indiscriminate use of antibiotics by humans in their different projects resulted in a profound transformation of bacteria themselves. She also shows how lateral/horizontal gene transfer, in which phages take an important role, has long been underestimated in the analysis of the rise of AMR. The antibiotics industry is based on exploiting some of the properties of microbes: these chemical molecules are produced naturally to mediate relations between microbial populations. By extracting the microbes that synthesise these molecules from their original environment and putting them to work in fermentation tanks, humans have generated quantities of antibiotics that are unprecedented in the history of the Earth, then poured them into environments in which these molecules did not necessarily belong, causing major disruption to these environments, with consequences that we now know are and will be disastrous (59). AMR as such, is a historically situated incarnation of pluribiosis.

But the history of the industrialisation of antibiotics has also produced material, epistemological and ontological infrastructures (60, 61), and thus constitutes the matrix in which and from which innovations around microbes are developed (62). Antibiotics are not just miracle drugs. They have also transformed marketing techniques and advertising (63), the entire pharmaceutical industry, evidence-based medicine (64), the behaviour of medical practitioners (e.g. basing diagnoses on symptoms and potential aetiologic agents), public health, animal husbandry and the agri-food sector (65). Moreover, antibiotics are now a phenomenal source of chemical pollution, reproducing and circulating (66). So, while antibiotics have led to a significant improvement in the health of human populations, the rise of bacterial resistance to antibiotics has revealed the flaws, limits, and lack of thought in the use of these living beings: microbes (and more globally living beings enlisted in human projects) inevitably act and react to attempts aiming at controlling or eradicating them. Moreover, microbes circulate within and between environments, spreading the resistance mechanisms from one ecosystem to the other.

The history of the industrialization of antibiotics is of importance when it comes to thinking the development of phages as anti-infective agents. First, because the infrastructural legacies of the industrialisation of antibiotics extend to the regulatory and epistemic frameworks structuring how any anti-infective agent should act and how its efficacy should be assessed. The very tangible risk then is to use phages as an *ersatz* of antibiotic molecules (67, 68), when their mode of action is slightly different and can offer, as we saw above, various possibilities based on their interaction capacities. Second, because if AMR is a reminder of the endless potentialities of living organisms and of our inability to predict with certainty the consequences of their uses, we have to bear in mind that phages are the most abundant biological entity on the planet (69, 70) and can be ubiquitous and diverse. Any use of them could thus have important consequences.



While phages are routinely used in molecular biology from the 1940s onwards (71), in therapy and in typing for at least the 1920s onwards (72, 73, 74), phage ecology as a discipline only emerged in the early 1990s. Despite this late start, there is already a wealth of data available, enabling us to assess the roles played by these viruses in different environments. Their local and global biogeography has been studied in different environments, from ocean regions to mammalian guts (75, 76, 77). Wilhelm and Suttle described in 1999 a complex mechanism they called the "viral shunt": every second, approximately  $10^{23}$  viral infections take place in the ocean, leading to the death of a number of infected hosts, from bacteria to whales, and thus influencing the composition of marine communities (78). More specifically, bacteriophage viruses are major players in the mortality of marine bacteria and act as "worlds composters" (79) by transforming living organisms into dissolved organic matter and making available the nutrients (particularly carbon and nitrogen) of which they were composed. The 'viral shunt' is therefore of vital importance for marine ecosystems and, more generally, biogeochemical cycles, including the carbon cycle. Phages can play crucial roles in trophic chains and might also be important players in soils microbiota (80).

Phages' role as composters is mirrored by their role as genetic proliferators. 35 to 50% of bacteria are susceptible to several (often with lysogenic cycle) phages. Evidence of multiple phages per host indicate that co-infections can occur, making host-virus interactions even more complex, with different consequences for ecology and evolution of both phages and bacteria. These encounters, exchanges, mixtures and assimilations occur with high frequency (81) and constitute an incredible force in the evolution of phages, bacteria and, potentially, other species, a force that biologists are only just beginning to grasp: despite the constant discovery of new genes thanks to the unprecedented development of genetic sequencing, particularly metagenomics, the vast majority of phage genomes remain unknown, as do the genes they carry and the functions associated with them. Bacteriophage viruses therefore constitute the largest reservoir of unknown genes on earth, a "Dark Matter" (82) leading to so many unimaginable possibilities. Human knowledge thus remains very partial regarding the richness and complexity of microbial life and the way humans can use or control it.

This should not be a brake on human projects, just an additional but mandatory precaution: to bear in mind both what scientists know and what they don't know, the existing infrastructures (what they allow and the way they constrain innovation), the pluribiotic skills of microbes and the multiple entanglements with the environments and milieus they inhabit and contribute to maintain and change.

### 3 - Vine and wine microbes

Let's take one situated example to illustrate this latest point, not in human health but in biocontrol, and more precisely in wine production, where phages could be used in a variety of ways, in order to show the various and intertwined factors, relations and scales that should be taken into account while trying to build and implement the use of any biological agent.

The culture of the vine is one of the oldest and most important agricultural crops in the world. Table grapes are sold for fresh consumption, while some other varieties are used for the

production of dried grapes, juices and wines. In 2020, the world area under vines, corresponding to the total surface area planted with vines for all purposes was estimated at 7.3 Mha (83). The Bordeaux region in France is one of the world's top wine regions. Experts report that it is warming up at an alarming rate. Even though changes have first contributed to increases in the average wine quality, fruit-based metrics now raise concerns that we are approaching a tipping point in traditional winegrowing (84). This belief is shared in other territories. Considering that a major redistribution of winegrowing regions would be catastrophic for numerous regional economies and ecologies in Europe, there's an urgency to reevaluate all practices to adapt and mitigate the induced phenomena. In the vineyard, winegrowers have therefore to adjust various parameters, ranging from variety selection, soil cultivation, irrigation, cultivation work and plant protection to mitigate the effects of climate change, establish systems that make cultivation sustainable and improve the quality of grape production, which is not without consequences for microbial populations, which are also crucial for the winemaking process and play an important role in the definition of wine characters (85). Managing adaptation is therefore also required during vinification and includes novel oenological practices.

Considering all above-mentioned issues, the world microbiology community was urged to pay particular attention to the microbiota dynamic from vineyard to cellar. As a result, inventories were undertaken in vineyards and in wineries. Even though such studies are becoming more comprehensive, using a wider range of technologies to collect information for a broader range of topics than was previously acquired, a review of the literature shows that the current investigations leave little and unequal room for phage ecology thinking. Examples are given below.

The development of next-generation DNA sequencing platforms, and integration of data from diverse omics approaches have facilitated the exploration of the complexity and functionality of the plant-associated microbial communities and showed that for something considered as a single entity, the vine, there are actually distinct and complex microbial communities associated with the rhizosphere, phyllosphere, leaf and root endospheres, as well as soil samples from cultivated grapevine (86, 87). There is no such thing as "the vine" from a microbiological point of view, but multiple ecosystems, each of which coming with its own specificities, its own microgeohistories. There is consequently a new awareness emerging of the need for a sustained drive to better capture the functioning and understand the biotic interactions that shape the dynamics of the multiple grapevine microbiota. Even though evidences about the crucial role of phages on ecosystem functions accumulate from other environments such as oceans, animal gut, or fermented foods, their presence in the microbiomes throughout plant lifetime have yet to be evaluated. These data are critical to address knowledge gaps on the roles of phages in the constant regulation of the diversity, richness, abundance, evolution, and physiology of microbial communities in natural environments.

And this all the more that all events and initiatives in the vineyard have a critical impact on the content of active substances in the fruit, which determines their taste and suitability for the winemaking process, making wine the result of entangled multispecies histories. The transformation of grapes into wine relies on yeasts and Lactic acid bacteria (LAB), which

drive the alcoholic and malolactic fermentations (AF and MLF), respectively. MLF is important in red winemaking and is increasingly used in white/sparkling wine production to reduce the sourness of malic acid and improve the aromatic complexity and microbial stability of wine. The main LAB species responsible for MLF is *Oenococcus oeni* (88, 89). The bacterium is better adapted to the limiting conditions imposed by the wine matrix and performs MLF under regular winemaking conditions, especially in wines with a pH below 3.5. Traditionally, both AF and MLF processes can occur spontaneously, and are initiated by microbial presence on grape skins and/or the cellar environment. However, the start, duration and qualitative impact of spontaneous MLF are unpredictable, which prompts winemakers to use pure starter cultures of selected bacteria to assure consistent wine flavour and quality. Due to the importance of *O. oeni* during winemaking, the presence of phages during winemaking has been early identified as a potential threat for the bacterium and the management of MLF (90). Accordingly, cases of wines with stuck MLF and high phage titers ( $>10^7$  PFU/ml) have been reported. It was later found that phages of *O. oeni* (oenophages) are frequently found at all steps of the process. Particles have been successfully isolated from all wine styles and chemical compositions worldwide, and those obtained from red wines exhibited higher diversity. A few strictly virulent phages have been isolated and are more frequent at the early steps (crushed grapes, musts) (91). At later steps, phage populations in wine reflect the spontaneous induction from lysogens, and the latter dominate the indigenous population upon completion of MLF (92). Intriguingly, presence of prophages was also found amongst commercial MLF strains. The question is then to know whether true benefits can be due to prophages at some stages, and whether lysogenization of selected strains may represent a leverage to improve the robustness of *O. oeni* and select novel starters. In addition to providing solutions to stakeholders, current investigations on phage-host interactions offer a comprehensive way of viewing the biology of *O. oeni*. They will provide insights into cell wall dynamics, mobile elements with regard to phage defense systems and their horizontal transfer through transduction, architecture of the genomes, and domestication to specific niches. The data available then show the high complexity of vine and wine multiple ecologies, the various and rich microbial interactions we are only beginning to apprehend and understand and, in these stories, the roles and functions of phages.

Bearing all this in mind, an interesting and open question is whether virulent phages may be used to limit undesirable bacteria in viticulture and wine production. In the vineyard, many serious and devastating diseases of grapevine worldwide are due to cryptogamic diseases. Yet, some can also be due to bacterial pathogens. One of the most feared bacterium is the xylem-restricted *Xylella fastidiosa*, the causal agent of Pierce's disease, which has circulated through California's vineyards since its introduction from Central America in the 1800s. Phage-based biocontrol protocols have been implemented *in planta* in Texas, USA, to control the pathogen (93). In the vineyard, the pathogen is vectored by an insect (the glassy-winged sharpshooter, *Homalodisca vitripennis*) which has also the capacity to uptake *X. fastidiosa* phages (94). Other bacterial pathogens include *Xylophilus ampelinus*, the causal agent of blight and canker on grapevine. Current studies aim to characterize the actual ecological niche of the bacterium (95).

Use of virulent phages may be also assessed to limit the development of bacterial spoilers in wines (LAB, acetic acid bacteria). Such bacteria produce off-flavours (mousiness, bitterness, geranium notes, volatile acidity, oily and slimy texture, and overt buttery characters). Other faults include cosmetic problems (e.g. turbidity, viscosity, sediment and film formation). Some LAB spoilers can also affect the wholesomeness of wine by producing biogenic amines and precursors of ethyl carbamate. Spoilage risk management includes the routine addition of sulphite (SO<sub>2</sub>) during vinification. The antimicrobial and antioxidant activities of the compound preserve the chemical and microbiological quality of wine. Yet, its use is questioned since exposure to SO<sub>2</sub> can cause adverse clinical effects in sensitive individuals, while its employment is not always compatible with the production of high-quality wines, as the compound itself can depreciate the final product. Even though the International Organisation of Vine and Wine and the European Union have established maximum levels, other concerns emerge. First, less acidic wines are produced in the context of global warming, which reduces SO<sub>2</sub> efficiency. Secondly, societal demand for authenticity, organic farming, and limited intervention is increasing in the food industry and the wine industry is no exception. Today, more winemakers decide to reduce SO<sub>2</sub> levels in wine, or omit external addition during the process. In Bordeaux, SO<sub>2</sub> reduction was employed by 35% of organic winemakers in 2019 (25% in 2017-18) (96). Reduced SO<sub>2</sub> use should not sacrifice wine quality, pushing the sector to raise attention to thoughtful farming and winemaking practices. Advancements in science are now required to develop credible alternatives to SO<sub>2</sub> and solutions may arise from the combination of different options. Some are authorised, including the use of physical and chemical (e.g. egg lysozyme) treatments. Microbial-based tools are increasingly attractive and meet consumer demands for clean label products. Phage-mediated control of pre- and post-harvest using strictly virulent phages may be a solution. To date, preliminary experiments have sporadically been performed in alcoholic beverage and are hampered by the limited availability of virulent phages targeting spoilers isolated from wines and ciders. To our knowledge, only phages infecting wine acetic acid bacteria have been characterized and correspond to temperate members of the *Tectiviridae* (97). An effort would be to make to isolate relevant virulent phages infecting spoilers and assess their ability to induce significant lysis of the bacterial population under wine conditions. Relevant and specific research questions need to be tackled to assess their technical suitability as bio-preservatives agents. They include the possible inactivation of particles by low pH, interference of polyphenolic compounds with phage-host recognition and presence of prophages and resistance systems in indigenous strains. It is reasonable to consider the complementary study of lytic phage-derived enzymes called endolysins (98). Such enzymes are produced to rapidly degrade the cell wall of the phage-infected cell and release new viral particles. Endolysins are experiencing renewed interest as they are bactericidal to (mainly) Gram<sup>+</sup> bacteria upon extrinsic addition. Their effectiveness is demonstrated in the medical and veterinary setting. With rapid activity, high efficiency, synergistic activity with different antibacterial agents, low risk of resistance development and resistance to harsh conditions, endolysins could be among the best alternatives. Endolysins are structurally similar to the authorised egg lysozyme but may confer greater specificity with less side effects on the microbiota.

A review of knowledge on the microbiology of vines and wine shows the extent of the knowledge available, the variety and richness of microbial interactions, while placing them in the context of the multiple ecologies in which they participate. Microbial communities are dynamic and evolving, and our knowledge is no more than a snapshot, at a given time, of their complex relationships, of constantly evolving microgeohistories.

Global warming and the pollution generated by the use of chemical inputs such as sulphites, among others, are requiring changes in viticulture and winemaking practices, which also affect the future of these microbial populations. Making use of the pluribiotic capacities of microbes could lead to new responses that respect ecosystems.

However, the examples discussed show how academic study is in its earliest stages to (i) understand the roles of phages in the biology of the healthy vine holobiont and the quality of the grapes used for all purposes, and (ii) propose innovative solutions to maintain the production and quality of derived products.

They also point to the fact that, whatever the responses envisaged, they will have to be elaborated collectively, and take into account the demands emanating from civil society, existing infrastructures, current regulations, as well as national and international policies, because microbes, as the example of AMR shows us, integrate into their biology the histories and choices made by human societies.

## **Conclusion:**

Innovation never takes place in a vacuum. The potential uses of phages are no exception. Existing regulations, supply and production chains, public policies, market structuring, modes of governance and, more generally, existing infrastructures make up the environment, which is also dynamic and evolving, in which the uses of phages will be deployed. The other articles in this special issue highlight the wide range of knowledge, initiatives, national policies and scientific and research infrastructures that already exist.

Since phages can cause the death of bacteria, they are most often thought of and presented in the field of innovation as ersatz antibiotics, reminding us of the weight of existing infrastructures - in this case antibiotic infrastructures - and of certain ways of thinking that sometimes become so dominant that we think they are universal. Phage evolution and phage ecologies tell quite different stories, in which the situated embodiment of biologies, and the knowledge produced about them, is central. Phages could be used wherever antibiotics are used, in human and animal health, in biocontrol and in the agri-food industry. However, the ubiquity of phages, their diversity and their constantly evolving pluribiotic relationship with bacteria and the microbiota mean that we can offer alternative responses, with fundamentally different care and attention practices. They can be used for different purposes by (i) minimizing bacterial evolution toward bacteriophage- or chemical antibiotic-resistance as well as (ii) directing bacterial evolution toward chemical antibiotic-sensitivity. Moreover, they can be used in variable ways depending on the environment, the practices, the state of knowledge, the various interests of the people involved or the political will to support (or not) innovations in the field of phages. In the field of human health, where AMR is pervasive, recognition of pluribiosis has in recent decades led to a desire to develop *à la carte*

phage therapy, whereas a prêt-à-porter approach has long been preferred by regulatory agencies and private stakeholders (see article 5 in this issue).

Whatever responses are considered, they should not be massive and one-size fits all. What we learn from pluribiosis and microgeohistories is precisely the need for situated responses, which actively take into account the dynamic and evolutionary dimension of microbes, as well as practitioners habits and uses, national or supranational regulations and policies, and existing infrastructures, in order to design novel pathways. These answers should indeed be based on an important premise: any antimicrobial intervention - whether chemical, enzymatic or viral - can have unexpected downstream negative effects. But this is actually very exciting, because we are not looking for a single, universal solution, but for variable ways of dealing with specific, situated problems. Controlling *Xyllela fastidiosa* in the vineyards of California may not require the same type of solution, or even the same phages, as in the olive groves of Puglia. The use of phages in one context should not lead to the idea that they can be used in another, whatever the specifics of the problem at hand.

All the more so as the roles of phages in different ecosystems, whether multiple human or vine microbiota, are far from known, and are likely to evolve. Whether it's a question of viral shunt or dark matter, to mention just two examples, we can see that phages are world composers and gene changers, phenomenal evolutionary forces. So it's not so much that they can, but that they must lead to alternative models, to different care practices, attentive both to infrastructural constraints and to situated ecologies, if we want to avoid contributing to the disasters underway. Interdisciplinary initiatives, whether they be colloquia, workshops, opinion papers or formal research projects, are a good place to start.

## References:

1. Helmreich S. Alien Ocean, Anthropological voyages in microbial sea. University of California Press: California; 2008
2. Jacquet S., Baudoux AC, Desdevises Y, et al. Les virus marins. Quae: Paris; 2023.
3. Paxson H, Helmreich S. The Perils and Promises of Microbial Abundance: Novel Natures and Model Ecosystems, from Artisanal Cheese to Alien Seas. *Social Studies of Science*. 2014; 44:165–93.
4. Lorimer J. The probiotic planet: using life to manage life. The University of Minnesota Press: Minneapolis; 2020
5. Moelling S. Viruses: more friends than foes. World Scientific; 2016.
6. Méthot P-O, Alizon S. What is a pathogen? Toward a process view of host-parasite interactions. *Virulence*. 2014;5(8):775-85. <https://doi.org/10.4161/21505594.2014.960726>.
7. O'Malley M. Philosophy of microbiology. Cambridge University Press: Cambridge; 2014.
8. Gilbert S, Sapp J, Tauber A. A Symbiotic View of Life: We Have Never Been Individuals. *The Quarterly Review of Biology*. 2021;87(4):325-341.

9. Turner JS. Extended Phenotypes and Extended Organisms. *Biology and Philosophy*. 2004;19(3):327-52. <https://doi.org/10.1023/B:BIPH.0000036115.65522.a1>.
10. Schuch R, Fischetti VA. Detailed Genomic Analysis of the W $\beta$  and  $\gamma$  Phages Infecting *Bacillus Anthracis*: Implications for Evolution of Environmental Fitness and Antibiotic Resistance. *Journal of Bacteriology*. 2006;188(8):3037-51. <https://doi.org/10.1128/JB.188.8.3037-3051.2006>.
- 11 Modi SR, Lee HR, Spina CS, et al. Antibiotic Treatment Expands the Resistance Reservoir and Ecological Network of the Phage Metagenome. *Nature*. 2013;499(7457):219-22. <https://doi.org/10.1038/nature12212>
12. Enault F, Briet A, Bouteille L, et al. Phages Rarely Encode Antibiotic Resistance Genes: A Cautionary Tale for Virome Analyses. *The ISME Journal*. 2017;11(1):237-47. <https://doi.org/10.1038/ismej.2016.90>.
13. Edlin G, Lin L, Kudrna R.  $\lambda$  Lysogens of *E. Coli* Reproduce More Rapidly than Non-Lysogens. *Nature*. 1975;255(5511):735-37. <https://doi.org/10.1038/255735a0>.
- 14 Wick LM, Qi W, Lacher DW, et al. Evolution of Genomic Content in the Stepwise Emergence of *Escherichia Coli* O157:H7. *Journal of Bacteriology*. 2005;187(5):1783-91. <https://doi.org/10.1128/JB.187.5.1783-1791.2005>.
15. Wang X, Kim Y, Ma Q, et al. Cryptic Prophages Help Bacteria Cope with Adverse Environments. *Nature Communications*. 2010;1(1):147. <https://doi.org/10.1038/ncomms1146>
16. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, et al. Engineered Bacteriophages for Treatment of a Patient with a Disseminated Drug-Resistant *Mycobacterium Abscessus*. *Nature Medicine*. 2019;25(5):730. <https://doi.org/10.1038/s41591-019-0437-z>.
17. Allen RC, Pfrunder-Cardozo KR, Meinel D, et al. Associations among Antibiotic and Phage Resistance Phenotypes in Natural and Clinical *Escherichia coli* Isolates. *mBio*. 2017;8:e01341-17.
18. Mathieu A, Dion M, Deng L, et al. Virulent coliphages in 1-year-old children fecal samples are fewer, but more infectious than temperate coliphages. *Nature Communications*. 2020;11:1-12.
19. Meaden S & Koskella B. Exploring the risks of phage application in the environment. *Front Microbiol*. 2013 ;4 :358. [doi: 10.3389/fmicb.2013.00358](https://doi.org/10.3389/fmicb.2013.00358)
20. Labrie SJ, Samson JE & Moineau S. Bacteriophage resistance mechanisms. *Nat Rev Micro*. 2010;8:317-327
21. Samson JE, Magadán AH, Sabri M, et al. Revenge of the phages: defeating bacterial defences. *Nat Rev Micro*. 2013;11:675-687
22. Egido JE, Costa AR, Aparicio-Maldonado C, et al. Mechanisms and clinical importance of bacteriophage resistance. *FEMS Microbiology Reviews*. 2022;46:fuab048

23. Chevallereau A, Blasdel BG, Smet JD, et al. Next-generation “-omics” approaches reveal a massive alteration of host RNA metabolism during bacteriophage infection of *Pseudomonas aeruginosa*. *PLoS Genet*. 2016;12:e1006134
24. Brives C. Pluribiosis and the never-ending microgeohistories. In: *With Microbes* (Brives C; Rest M, Sariola S eds.) Mattering Press: London; 2021; pp. 247-267.
25. Gómez P, Buckling A. Bacteria-phage antagonistic coevolution in soil. *Science*. 2011;332(6025):106-9. doi: 10.1126/science.1198767. PMID: 21454789.
26. Pradeu T, Kostyrka G, Dupré J. *Understanding Viruses: Philosophical Investigations. Studies in History and Philosophy of Biological and Biomedical Sciences*. 2016;59:57-63
27. Forterre P. Manipulation of cellular syntheses and the nature of viruses: The virocell concept. *Comptes Rendus Chimie*. 2011;14(4) :392-99.  
<https://doi.org/10.1016/j.crci.2010.06.007>.
28. Rodríguez-Rubio L, Blanco-Picazo P, Muniesa M. Are Phages Parasites or Symbionts of Bacteria?. In: Witzany, G. (eds) *Biocommunication of Phages*. Springer Cham.  
[https://doi.org/10.1007/978-3-030-45885-0\\_7](https://doi.org/10.1007/978-3-030-45885-0_7)
29. Węgrzyn G. Should Bacteriophages Be Classified as Parasites or Predators? *Pol J Microbiol*. 2022;71(1):3-9. doi: 10.33073/pjm-2022-005. PMID: 35635166; PMCID: PMC9152906
30. Secor PR, Dandekar AA. More than Simple Parasites: the Sociobiology of Bacteriophages and Their Bacterial Hosts. *Mbio*. 2020;11(2). [doi.org/10.1128/mbio.00041-](https://doi.org/10.1128/mbio.00041-20)
31. Brives C. Pluribiosis and the never-ending microgeohistories. In: *With Microbes* (Brives C; Rest M, Sariola S eds.) Mattering Press: London; 2021; pp. 247-267.
32. Adli M. The CRISPR tool kit for genome editing and beyond. *Nature Communications*. 2018 ;9 :1911. <https://doi.org/10.1038/s41467-018-04252-2>
33. Kortright KE, Chan BK & Turner PE. High-throughput discovery of phage receptors using transposon insertion sequencing of bacteria. *PNAS*. 2020;117:18670-18679.
34. Mutalik VK, Adler BA, Rishi HS, et al. High-throughput mapping of the phage resistance landscape in *E. coli*. *PLOS Biology*. 2020;18:e3000877.
35. Adler BA, Kazakov AE, Zhong C, et al. The genetic basis of phage susceptibility, cross-resistance and host-range in *Salmonella*. *Microbiology*. 2021;167:001126.
36. Wright RCT, Ville-Petri F, Smith MCM, et al. Cross-Resistance Is Modular in Bacteria–Phage Interactions. *PLOS Biology*. 2018;16(10): e2006057.  
[doi.org/10.1371/journal.pbio.2006057](https://doi.org/10.1371/journal.pbio.2006057).



37. Bohm K, Porwollik S, Chu W, et al. Genes affecting progression of bacteriophage P22 infection in Salmonella identified by transposon and single gene deletion screens. *Molecular Microbiology*. 2018;108:288-305.
38. Christen M, Beusch C, Bösch Y, et al. Quantitative Selection Analysis of Bacteriophage  $\phi$ CbK Susceptibility in *Caulobacter crescentus*. *Journal of Molecular Biology*. 2016;428:419-430.
39. Cowley LA, Low AS, Pickard D, et al. Transposon Insertion Sequencing Elucidates Novel Gene Involvement in Susceptibility and Resistance to Phages T4 and T7 in *Escherichia coli* O157. *mBio*. 2018;9:e00705-18.
40. Cohan FM, Zandi M & Turner PE. Broad-scale phage therapy is unlikely to select for widespread evolution of bacterial resistance to virus infection. *Virus Evolution*. 2020;6(2):veaa060. doi: 10.1093/ve/veaa060. PMID: 33365149; PMCID: PMC7744382. Format:
41. Froissart R, Brives C. Evolutionary biology and regulation of medicines: a necessary 'pas de deux' for future successful bacteriophage therapy. *Journal of Evolutionary Biology*. 2021;34(12):1855-1866. <https://doi.org/10.1111/jeb.13904>
42. Bull JJ, Levin BR, Molineux IJ. Promises and Pitfalls of In Vivo Evolution to Improve Phage Therapy. *Viruses*. 2019;11(12). <https://doi.org/10.3390/v11121083>
43. Torres-Barcelo C, Turner P, Bucking A. Mitigation of evolved bacterial resistance to phage therapy. *Curr Opin Virology*. 2022;53:101201. doi.org/10.1016/j.coviro.2022.101201.
44. Appelmans. Le dosage du Bacteriophage. *Comptes Rendus de l'Académie de Sciences*. 1921:1098.
45. Burrowes BH, Molineux IJ, Fralick JA. Directed in vitro evolution of therapeutic bacteriophages: the Appelmans protocol. *Viruses*. 2019;11:241.
46. Maurin et al. in prep.
47. Mapes AC, Trautner BW, Liao KS, et al. Development of expanded host range phage active on biofilms of multi-drug resistant *Pseudomonas aeruginosa*. *Bacteriophage*. 2016;6:e1096995.
48. Peters TL, Song Y, Bryan DW, et al. Mutant and Recombinant Phages Selected from In Vitro Coevolution Conditions Overcome Phage-Resistant *Listeria monocytogenes*. *Appl. Environ. Microbiol*. 2020 ;86(22):e02138-20.
49. Sáez Moreno D, Visram Z, Mutti M, et al.  $\epsilon$ 2-Phages Are Naturally Bred and Have a Vastly Improved Host Range in *Staphylococcus aureus* over Wild Type Phages. *Pharmaceuticals*. 2021;14:325.
50. Torres-Barcelo C, Turner P, Bucking A. Mitigation of evolved bacterial resistance to phage therapy. *Curr Opin Virology*. 2022;53:101201. doi.org/10.1016/j.coviro.2022.101201.

51. Oechslin F. Resistance Development to Bacteriophages Occurring during Bacteriophage Therapy. *Viruses*. 2018;10(7):351. <https://doi.org/10.3390/v10070351>.
52. Seed KD, Minmin Y, Shapiro BJ, et al. Evolutionary consequences of intra-patient phage predation on microbial populations. *eLife*. 2014:e03497. <https://doi.org/10.7554/eLife.03497>.
53. Chan BK, Siström M, Wertz JE, et al. Phage Selection Restores Antibiotic Sensitivity in MDR *Pseudomonas Aeruginosa*. *Scientific Reports*. 2016;6: 26717. <https://doi.org/10.1038/srep26717>.
54. Chan BK, Turner PE, Kim S, et al. Phage Treatment of an Aortic Graft Infected with *Pseudomonas Aeruginosa*. *Evolution, Medicine, and Public Health*. 2018;1:60-66. <https://doi.org/10.1093/emph/eoy005>.
55. Gurney J, Brown SP, Kaltz O, et al. Steering Phages to Combat Bacterial Pathogens. *Trends in Microbiology*. 2020;28(2):85-94. <https://doi.org/10.1016/j.tim.2019.10.007>.
56. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, et al. Engineered Bacteriophages for Treatment of a Patient with a Disseminated Drug-Resistant Mycobacterium Abscessus. *Nature Medicine*. 2019;25(5):730. <https://doi.org/10.1038/s41591-019-0437-z>.
57. Pirnay JP. Phage Therapy in the Year 2035. *Frontiers in Microbiology*. 2020;11:1171. <https://doi.org/10.3389/fmicb.2020.01171>.
58. Landecker H. Antibiotic resistance and the biology of history. *Body & Society*. 2016;22(4):19-52.
59. Brives C. *Pluribiose: travailler avec les microbes*. Quae: Paris; 2024.
60. Chandler C. *Current Accounts of Antimicrobial Resistance: Stabilisation, Individualisation and Antibiotics as Infrastructure*. Palgrave Communications. 2019;5:53.
61. Brives C, Pourraz J. Phage therapy as a potential solution in the fight against AMR: obstacles and possible futures. *Palgrave Communications*. 2020;6(1):1-11.
62. Brives C. *Face à l'antibiorésistance: une écologie politique des microbes*. Editions: Paris Amsterdam; 2022.
63. Podolsky S. *The Antibiotic Era. Reform, resistance, and the pursuit of a rational therapeutics*. Johns Hopkins University Press: Baltimore; 2015.
64. Podolski S. Antibiotics and the Social History of the controlled clinical trial, 1950-1970. *Journal of the History of Medicine and Allied Sciences*. 2010;65(3):327-367.
65. Kirchhelle C. *Pyrrhic Progress, the history of antibiotics in Anglo-American food production*. Rutgers University Press: New Brunswick; 2020.

66. Gillings M. Evolutionary Consequences of Antibiotic Use for the Resistome, Mobilome, and Microbial Pangenome. *Frontiers in Microbiology*. 2013;4(4).
67. Brives C, Pourraz J. Phage therapy as a potential solution in the fight against AMR: obstacles and possible futures. *Palgrave Communications*. 2020;6(1):1-11.
68. Pirnay JP, De Vos D, Verbeken G et al. The Phage Therapy Paradigm: Prêt-à-porter or Sur-mesure? *Pharmaceutical Research*. 2011 ;28 :934-937.
69. Fuhrman J. Marine viruses and their biogeochemical and ecological effects. *Nature*. 1999;399(6736):541–548; doi: 10.1038/21119.
70. Suttle CA. Marine viruses - major players in the global ecosystem. *Nat Rev Microbiol* 2007;5(10):801–812; doi: 10.1038/nrmicro1750.
71. Kay L. *The molecular vision of life*. Oxford University Press: Oxford; 1993.
72. Summers W. The Strange History of Phage Therapy. *Bacteriophage*. 2012;2(2): 130-133.
73. Ansaldi M, Boulanger P, Brives C, et al. Un siècle de recherche sur les bactériophages. *Virologie*. 2020;24(1):9-22. doi:10.1684/vir.2020.0809
74. Kirchhelle C. The forgotten typers: The rise and fall of Weimar bacteriophage-typing (1921–1935). *Notes and Records*. 2020;74(4):539-565
75. Shkoporov AN, Stockdale SR, Lavelle A, et al. Viral biogeography of the mammalian gut and parenchymal organs. *Nat Microbiol*. 2022;7(8):1301–1311; doi: 10.1038/s41564-022-01178-w.
76. Thurber RV. Current insights into phage biodiversity and biogeography. *Curr Opin Microbiol*. 2009;12(5):582–587; doi: 10.1016/j.mib.2009.08.008.
77. Clokie MRJ, Millard AD, Letarov AV, et al. Phages in nature. *Bacteriophage*. 2011;1(1):31–45; doi: 10.4161/bact.1.1.14942.
78. Wilhelm SW, Suttle CA. Viruses and Nutrient Cycles in the Sea. *Bioscience*. 1999;49(10):781-788.
79. Brives C. *Face à l'antibiorésistance, une écologie politique des microbes*. Éditions Amsterdam: Paris; 2022.
80. Fierer, N. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat Rev Microbiol*. 2017;15:579–590.
81. Pfeifer E, Sousa JM, Touchon M., et al. When bacteria are phage playgrounds :interactions between viruses, cells, and mobile genetic elements. *Current Opinion in Microbiology*. 2022;70:102230.
82. Hatfull G. Dark Matter of the biosphere: the amazing world of bacteriophage diversity. *Journal of Virology*. 2015;89:16.

83. International Organisation of Vine and Wine. Annual Report. 2021.
84. Gambetta GA, Kurtural SK. Global warning and wine quality: are we close to the tipping point ? *OENO One*. 2021;55:353-361.
85. Tempère S, Marchal A, Barbe JC, et al. The complexity of wine: clarifying the role of microorganisms. *Appl Microbiol Biotechnol*. 2018 ;102(9):3995-4007. doi:10.1007/s00253-018-8914-8
86. Bettenfeld P, Cadena I, Canals J, et al. The microbiota of the grapevine holobiont: a key component of plant health. *Journal of Advanced Research*. 2022;40:1-15.
87. Fournier P, Pellan L, Barroso-Bergadà D, et al. The functional microbiome of grapevine throughout plant evolutionary history and lifetime. *Advances in Ecological Research*. 2022;67:27-99.
88. Grandvalet C. *Oenococcus oeni*: Queen of the Cellar, Nightmare of Geneticists *Microbiology*. 2017;163:297–299.
89. Tempère S, Marchal A, Barbe JC, et al. The complexity of wine: clarifying the role of microorganisms. *Appl Microbiol Biotechnol*. 2018 ;102(9):3995-4007. doi:10.1007/s00253-018-8914-8
90. Chaïb A, Philippe C, Jaomanjaka F, et al. Phage-host interactions as a driver of population dynamics during wine fermentation: Betting on underdogs. *International Journal of Food Microbiology*. 2022;383:109936.
91. Philippe C, Krupovic M, Jaomanjaka F, et al. Bacteriophage GC1, a Novel Tectivirus Infecting *Gluconobacter cerinus*, an Acetic Acid Bacterium Associated with Wine-Making. *Viruses*. 2018;10(1):39. doi:10.3390/v10010039
92. Chaïb A, Philippe C, Jaomanjaka F, et al. Phage-host interactions as a driver of population dynamics during wine fermentation: Betting on underdogs. *International Journal of Food Microbiology*. 2022;383:109936.
93. Das M, Bhowmick TS, Ahern SJ, et al. Control of Pierce's disease by phage. *PLoS One*. 2015;10(6):e0128902.
94. Clavijo-Coppens F, Ginet N, Cesbron S, et al. Novel virulent bacteriophages infecting mediterranean isolates of the plant pest *Xylella fastidiosa* and *Xanthomonas albilineans*. *Viruses*. 2021;13:725.
95. Portier P, Taghouti G, Bertrand P-E, et al. Analysis of the Diversity of *Xylophilus ampelinus* Strains Held in CIRM-CFBP Reveals a Strongly Homogenous Species. *Microorganisms*. 2022 ;10(8):1531. doi.org/10.3390/microorganisms10081531.
96. Pelonniér-Magimel E, Mangiorou P, Darriet P, et al. Sensory characterisation of Bordeaux red wines produced without added sulphites. *OENO One*. 2020;54:733-743.

97. Philippe C, Krupovic M, Jaomanjaka F, et al. (2018) Bacteriophage GC1, a Novel Tectivirus Infecting *Gluconobacter cerinus*, an Acetic Acid Bacterium Associated with Wine-Making. *Viruses*. 2018 ;10(1):39. doi:10.3390/v10010039
98. Schmelcher M & Loessner MJ. Bacteriophage endolysins: applications for food safety. *Curr Opin Biotechnol*. 2016;37:76-87.