ACTA MICROBIOLOGICA BULGARICA Volume 40 / 2 (2024)

Review

Phage Therapy and Phage Biocontrol – between Science, Real Application and Regulation

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Abstract

The increasing emergence of antibiotic-resistant bacteria is considered a "silent epidemic", globally, and requires urgent action in the development of new effective treatments for severe bacterial diseases. Regarding this, one of the most promising approaches, that is gaining favor both in the scientific community and in the real world of applied medicine is the use of phages, the naturally occurring killers of bacteria, as supplements or substitutes for traditional disease therapies. The possible ways of application of phages in the combat with resistant bacteria are quite diverse: as integrated plant protection strategies in agriculture and as substitutes for chemical pesticides, in food safety, in aquatic systems, and animal and human healthcare. However, certain challenges shall be highlighted in terms of the advancement of studies on the potential of phages, as well as the regulatory framework controlling the manufacture and implementation of phage-based products. Moreover, the general understanding of the use of bacteriophages as antibacterial agents needs further clarification among the public. Thus, it is crucial to promote phage research, particularly in nations with little or no experience with phage studies as therapeutic agents. In this regard, in light of the new opportunities in the fight against multidrug-resistant bacteria, this review aims to take a look at the phages, the natural killers of bacteria.

Keywords: phage therapy, phage biocontrol, regulation, phage endolysins, multidrug-resistant bacteria

Резюме

Нарастващият брой на резистентни на антибиотици бактерии се счита за „тиха епидемия", в световен мащаб, и изисква спешни действия в разработването на нови ефективни лечения на тежките бактериални заболявания. В тази връзка, един от най-обещаващите подходи, който печели все по–голяма популярност както в научната общност, така и в реалния свят на приложната медицина, е използването на бактериофаги, като добавки или заместители на традиционните терапии на бактериалните заболявания. Възможните начини за приложение на фагите в борбата с резистентните бактерии са доста разнообразни: като интегрирани стратегии за растителна защита в селското стопанство и като заместители на химическите пестициди, в безопасността на храните, във водните системи и в здравеопазването на животните и хората. Съществуват обаче определени предизвикателства, които могат да бъдат подчертани по отношение на напредъка в проучванията на потенциала на фагите, както и по отношение на регулаторната рамка, контролираща производството и прилагането на продукти, базирани на фаги. Освен това, общото разбиране за употребата на бактериофаги като антибактериални агенти се нуждае от допълнително разясняване сред обществеността. Поради това е от решаващо значение да се насърчават изследванията върху фагите, особено в страни с малък или никакъв опит в проучванията на фаги като терапевтични средства. В тази връзка, в светлината на новите възможности в борбата с мултирезистентните бактерии, този обзор има за цел да хвърли поглед към фагите, естествените убийци на бактериите.

Introduction

Viruses are considered the most abundant biological forms of life on our planet. It has been proposed that their quantity is near 10^{31} (Breitbart and Rohwer, 2005). Sequencing analyses of collective viral genomes in various environmental samples showed that the greater part of this viral community

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Acta Microbiol. Bulg. 2024; 40(02). https://doi.org/10.59393/amb24400204

falls on bacteriophages - viruses that kill bacteria (Breitbart and Rohwer, 2005). The role of phages, as they are called in short, in nature, is as natural predators of bacteria, controlling their quantity. Usually, phages have targeted action as they can kill a particular bacterial species, even some of its strains or, in some cases, closely related species from different genera (Pantucek *et al*., 1998; Paolozzi and Ghelardini, 2006; Gupta and Prasad, 2010). Such phages are known as narrow host range, broad host range, or polyvalent, respectively (Ross *et al*., 2016).

The increasing number of antibiotic - resistant bacteria and the occurrence of the so - called "super bugs" are the driving force behind the discovery and development of new effective treatments for severe bacterial diseases (São-José *et al*., 2022). The World Health Organization was alarmed by this increasing concern in a report to the secretary-general of the United Nations in April 2019 (IACG, 2019). In this regard, the use of phages as alternatives or additives to conventional disease treatments is considered needed and urgent. The possible ways of applying phages in the combat with resistant bacteria are quite diverse: as integrated plant protection strategies in agriculture and as substitutes for chemical pesticides (Buttimer *et al*., 2017; Svircev *et al*., 2018; Holtappels *et al*., 2021), in food safety (Garcia *et al*., 2023), in aquatic systems (Ahiwale *et al*., 2012), and in animal and human healthcare. Moreover, phages possess several key advantages over antibiotics and chemical compounds: 1/ the evolutionary pressure over bacteria and the dispersal of resistance among a large number of bacteria is less due to their targeted action in comparison to the broad-spectrum action of antibiotics and chemicals (Loc-Carrillo *et al*., 2011); 2/ it has been noted that phage resistance might decrease the pathogenicity of bacteria (Levin *et al*., 2004; Capparelli *et al*., 2010).

Treatment of severe bacterial diseases with bacteriophages is known as 'phage therapy'. However, 'phage biocontrol' rather than 'phage therapy' is the correct term when it comes to phage application in agriculture (Buttimer *et al*., 2017). Actually, the application of phage therapy is well-known in Eastern Europe (Georgia, Poland, and Russia). However, in the rest of Europe, this approach is still neglected, although the interest of the responsible institutions has been growing in the last few years. However, there is still no officially approved phage product in the European Union or in the United States market (Pirnay and Verbeken, 2023).

In light of the new opportunities in the fight against resistant bacteria, this review aims to take a look at phages, the natural killers of bacteria. The review will emphasize the history of their discovery, their diversity concerning the life cycle, the urgent need for more in-depth studies, reveling their potential as substitutes for antibiotics and chemical pesticides, the possible application of phage endolysin products instead of viable phages, and finally, the problems with regulation of phage products in Europe.

A look at the past

It is assumed that the very first supposition about the presence of phages in living nature appeared somewhere around 1896 when Ernest Hankin observed anti-*Vibrio cholerae* action of the water from the rivers Ganges and Jumna (Huang *et al*., 2022). These assumptions served as the basis for the following years of phage discovery. The term "bacteriophage" is a combination of two words and means "bacteria eater". The actual discovery of phages dates back to the beginning of the twentieth century and is linked with the names of Felix d'Herelle and Frederick Twort (Twort, 1915; d'Herele, 1917). They first suggested that bacteriophages are actually viruses (Twort, 1936; Buttimer *et al*., 2017). Then, the role of these bacteria "eaters" as antibacterial agents in the treatment of patients with dysentery was clearly demonstrated by d'Herelle during his surveys in the Hospital des Infants – Malades (Wilkinson, 2001). His revolutionary findings stimulated other researchers to explore the potential of phages in the treatment of other severe human infections such as staphylococcal, bubonic plaque, and cholera (Sulakvelidze *et al*., 2001). Besides this, the potential of phages in the fight against bacterial plant diseases has also been studied. Such pivotal studies have been reported by Mallmann and Hemstreed (1924), Coons and Kotila (1925), and Kotila and Coons (1925). In these studies, the role of phages as antibacterial agents has been demonstrated against the causative agents of cabbage rot (*Xanthomonas campestris* pv. *campestris*) and soft rot on potatoes and carrots (*Pectobacterium atrosepticum* and *P. carotovorum* susp. *carotovorum*, respectively). Several years later, in 1935, Thomas conducted the first trial under real field conditions and demonstrated that seed treatment with phages against the phytopathogenic bacterium *Pantoea stewartii* (Steward's disease), reduced the disease severity to 1.5%, compared to 18% of untreated plants (Thomas, 1935).

However, parallel with these findings, sci-

ence has also progressed in terms of the discovery of various antibacterial substances, antibiotics (AB), synthesized by microorganisms, mainly molds. The historical data of antibiotic discovery dates back to the nineteen centuries, when Sir John Scott Burden-Sanderson observed that when broth media is covered with molds, no bacterial growth was detected. In 1871, Joseph Lister established the antibacterial effect of *Penicillium glaucum.* Several years later, in 1877, Luis Pasteur and colleagues observed that the bacterium *Bacillus anthracis* growth was suppressed during cultivation with other aerobic bacteria. Almost a decade later, Jean-Paul Vuillemin defined „antibiosis“ as any biological interaction in which „one living organism kills another to ensure its own existence“. However, until that time, no active antibiotic molecule has been obtained in pure form (Durand *et al*., 2019). The first commercial antibiotic, named Salvarsan®, was produced in 1911 form arsphenamine, a chemical derivate, active against the lues causative agent *Treponema pallidum*. Its discoverer was Paul Ehrlich. Later, in 1935, the antibiotic Prontosil®, which has the active substance sulphanilamide, was also produced and used during World War II (Durand *et al.,* 2019). Soon after these findings, in the 1940s began the "golden age" of antibiotics with the industrial production of penicillin, isolated from *P. chrysogenum* (Fleming, 1945). The antibacterial effect of penicillin was established accidentally by Alexander Fleming in 1928, but its active molecule was obtained and purified several years later, in 1940, by Howard Flory and Ernst Chain (Fleming, 1945). This "period" lasts almost 40 years with the discovery and commercialization of more than 40 antibiotics (Gordillo Altamirano and Barr, 2019). During this time, the observed antibiotic resistance (ABR), a consequence of overuse, was not a concern because new and effective AB substances were quickly produced and introduced into the market. However, during the 1990s, studies on the discovery of new AB substances slowed considerably, and practically all new AB preparations were either altered or combination forms of already approved substances (Gordillo Altamirano and Barr, 2019). Moreover, the extensive and uncontrolled use of AB and the evolutionary pressure on the bacteria as a consequence of this resulted in the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) pathogenic bacteria (Magiorakos *et al*., 2012). Finally, the bacterial ABR was considered by the World Health Organization (WHO) as one of the most serious threats to humanity (Shrivastava *et al*., 2018). In this regard, studies of new and effective manners in combat with MDR, XDR, and PDR are highly encouraged and, in some sense, urgent. One of the most promising approaches in this combat is the use of phages as alternatives/substitutes for known AB (Hatfull *et al*., 2022).

Phage replication cycles

Bacteriophages possess two primary ways of reproduction: lytic and lysogenic replication cycles. Lytic phages have a replication cycle that ends with bacterial death. During this type of phage infection, all phages' structural elements are produced inside the bacterial cell using its synthetic machinery. The phage genome is replicated and packed in new viral particles. The final step of this cycle is linked with the production of hydrolytic enzymes (endolysins or murein hydrolases) which degrade the bacterial cell wall from inside. This step is crucial for the release of the newly formed phage particles outside the cells which, in the end are destroyed. In terms of phage therapy and the use of phages as therapeutic agents, obligate lytic phages are those who matter. On the other hand, the lysogenic phages do not destroy the host cell upon phage infection. Those phages are also known as temperate phages. When the phage inserts its genetic material into the bacterial cell, it incorporates it into its genome. This specific condition is considered a latent form of the phage and is known as "prophage". Prophages replicate along with the bacterial host genome and can switch into a lytic cycle under specific conditions, i.e., damage to the genetic material (Feiner *et al*., 2015). Because lysogenic phages do not always destroy the bacterial host cell, they have no such value as therapeutic agents, with some exceptions. It is possible for a temperate phage to be genetically manipulated concerning the removal of the repressor genes, thus obtaining lytic derivatives (Debrick *et al.,* 2019; Hatfull *et al*., 2022). Such manipulation would be required only in cases where no obligate lytic phages are available to threaten the given targeted pathogenic bacteria. Such a case has been described with engineered lysogenic phages to threaten cystic fibrosis infection caused by drug-resistant *Mycobacterium abscessus*, in a 15-year-old patient. (Debrick *et al*., 2019). Besides these two main ways of phage replication, there is another that is much less often described in the literature: the pseudolysogenic replication cycle (Feiner *et al*., 2015). In this situation, the phage genome fails to integrate into the bacterial host genome, and at the same time, fails to enter a lytic cycle. Actually, the

phage genome exists in the bacterial cytoplasm as an independent structure, called pre-prophage. This condition is commonly associated with cell starvation, which occurs when bacteria are unable to conduct cellular processes such as DNA replication and protein synthesis. When nutrients are available to the bacterial cell, these pre-prophages can implement either lytic or lysogenic replication cycles. It is important to highlight that when a phage persists as a pre-prophage, it does not replicate alongside the host cell chromosome and eventually resides in one of the daughter cells following cell division (Feiner *et al*., 2015).

Considering phages for using as antibacterial agents

One thing is sure: there will always be bacteriophages in the environment, and they are practically an inexhaustible resource. Thus, phages could be considered a cure given "from nature to nature", meaning that the solution to problems that arise from nature, i.e., resistant bacteria, could also be found in nature – the phages. All we need to do is find them, isolate and characterize them, and mobilize their potential to work for us. As natural antagonists of bacteria, bacteriophages have great potential as antibacterial agents and could be used in combat with severe bacterial diseases in humans, animals, plants, aquacultures, as food supplements, etc. (Chibeu *et al*., 2013; Diana *et al*., 2017; Grant *et al.,* 2017; Zhang *et al*., 2019; Zbikowska *et al.,* 2020; Ji *et al*., 2021).

It is known that phages are everywhere around and inside us and can be found in particularly all-natural niches. The presence of a given phage in the corresponding location depends on the existence of the respective host bacteria (Hyman *et al*., 2019). For example, phages affecting phytopathogenic bacteria could be found in the phyllosphere and rhizosphere of the plants (Buttimer *et al*., 2017; Akbaba and Ozaktan, 2021; Kizheva *et al*., 2021, 2023; Holtappels *et al*., 2022;), phages for enterobacteria and fecal enterococci could be found in sewage, wastewater and directly in feces (Jensen *et al*., 1998; Jakhetia *et al*., 2013, Karumidze *et al*., 2013, Li *et al.,* 2016, Townsen*et al*., 2021; Cieślik *et al*., 2022; Ali *et al*., 2024), phages affecting skin pathogenic bacteria can be found on skin (Farrar *et al*., 2007; Liu *et al*., 2015), throat samples from healthy people (Ronda *et al*., 1981) or from wound secretions (Rasool *et al*., 2016). However, the isolation of suitable phages is just the beginning of the process of establishing effective preparation. Several key phage features must be considered before a phage is chosen as a therapeutic agent. Therapeutic phages should be obligately lytic (with some exclusions), have a long – term survival rate at different storage temperatures, be easily propagated in high titers, and have a broad host range, i.e., can destroy a large number of bacterial strains, the genome of the phage should not carry any genes for known or suspected toxins, and the phage should not be able to transfer genes from one bacterium to another, i.e., to act as vector in transduction processes. Beyond these general requirements, phages intended for use in agriculture should meet several additional criteria. It is important to determine phages' susceptibility to chemical pesticides. For example, it has been reported that chemical pesticides may contain various substances that can affect phage activity (Yamamoto *et al.,* 1968; Chattopadhyay *et al*., 2002). The negative influence of UV light on phage viability should also be considered, as phages are mainly applied as foliar preparations (Buttimer *et al.,* 2017). The influence of several other factors on phage viability should also be determined: pH, temperature, plant metabolites, etc. (Erskine *et al*., 1973; Delitheos *et al.,* 1997; Iriarte *et al*., 2007). Concerning phages applied to humans and animals, there are some obstacles that should also be noted. It has been reported that in some cases, phages' activity *in vivo* (in a human or animal body) could be decreased due to not clearly understood circumstances. Thus, more in-depth studies on phages' effectiveness *in vivo* should be performed to clarify the factors that could inhibit their lytic actions (Hyman *et al.,* 2019; Hatfull *et al.,* 2022).

Beyond science – lab phage research and released products for phage therapy and biocontrol

Understanding the urgent need for innovative and effective therapeutics for fighting MDR, XDR, and PDR bacteria, it is important to ask ourselves a question: what do we have to do to boost the process of implementing phage therapy globally? One possible answer is to join our efforts in searching, isolating, and characterizing diverse phages that can be used as therapeutic agents. Also, it is important that information about our phages, especially those that could help a specific urgent need, be shared. One of the most common manners for sharing detailed phage characteristics is to publish the obtained results from phage research in a scientific paper. However, there is another way to share our findings, to ask questions, to collaborate with colleagues working in the field, and to stay up-to-date with the current needs for help to treat

urgent infections, worldwide. This place is called Phage Directory (Tay, 2023). Established in 2017 by Jessica Sacher and Jan Zheng, the directory has a clear mission: to build a bridge between phage researchers, academic laboratories, companies, phage therapy centers, doctors, and patients globally. Unfortunately, there are still a limited number of listed phage laboratories, 180, in comparison to the thousands of labs working on other disease treatment developments (Tay, 2023). As antimicrobial resistance is increasing very fast, we hope that the number of laboratories sharing their phages for global use, will increase too.

The issue of phage applications as alternatives to antibiotics is gaining more and more interest, not only among researchers but also among other interested parties such as companies and policymakers. Recently, a meeting (ECOPHAGE) focused on the possibility of using phages in eco-sustainable agriculture and food systems, was held at the Universitat de Valencia, Valencia, Spain from September 12-13 2023. The discussed themes from that workshop are summarized by Garcia *et al*. (2023). The major outcomes from that meeting are that phages have the potential to be applied in the agriculture and food sectors either as treatment strategies or as prophylactics. However, it has been noted that there are some important things that need to be considered regarding phage application: optimizing phage formulations, choosing the best route of administration, using Artificial Intelligence in designing phage cocktails, screening for emerging phage – resistant bacteria, etc. The issue of phage safety assessment has also been discussed and the major conclusion was that it needs to be validated to meet the regulatory requirements, which are quite different depending on the current application. Finally, the potential of phages' endolysins as antibacterial substances is another promising solution, which needs to be more in-depth studied and potentially implemented in the therapy (Garcia *et al*., 2023).

Phages' potential in agriculture

The number of articles reporting on the isolation and characterization of phages with various applications has increased. Table 1 summarizes the data from the recently published papers, reporting phages that have the potential for biocontrol of various severe bacterial diseases on plants. The substantial number of published papers for the past 5-6 years indicates that the research interest in phage studies has grown significantly. However, the number of released phage products is still very small. Huang *et al*. have summarized the data concerning the available commercial products intended for use in agriculture: 1/ BioLyse®BP (against soft rot *Enterobacteriaceae*), 2/ AgriPhage CMM™ (against *Clavibacter michiganensis* subsp. *michiganensis*), 3/ AgriPhage Citrus canker (against *X. citri*), 4/ AgriPhage Fire Blight (against *Erwinia amylovora*), 5/ Erwiphage (against *E. amylovora*) and 6/ AgriPHIX (against diverse bacteria) (Huang *et al.,* 2022). This list can be supplemented with 7/ AgriPhage Spot and Speck (against *X. campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*); 8/ AgriPhage Nut and Stone Fruit (against *Xanthomonas arboricola* pv. *pruni, X. arboricola* pv. *juglandis, X. arboricola* pv. *corylina,* and *P. syringae* pv. *syringae).* Another company engaged in the production of phage-based products for agriculture is EcoPhage, founded in 2019 and based in Israel. Three products are available for the treatment of bacterial spot and speck on tomatoes (GoldenEco), fire blight on pears and apples (FireEco), and *Ralstonia solancearum* race 2 on bananas (EcoStonia) (https://ecophage.com/).

Phages' potential in human, veterinary, and food sectors

As those three sectors of phage application share threats from similar bacteria, they will be discussed together. The studies reporting data on phages with the potential for controlling human and animal pathogenic bacteria are much more than those described in Table 1. The reason is probably that the efforts of scientists are focused primarily on the application of such phages concerning the global concern: the growing antibiotic resistance among human and animal pathogenic bacteria. Just for example, the published papers focused on the study of *Escherichia coli* phages and their potential for phage therapy are more than 50 from the beginning of 2024. This proves that a lot of effort is being put into the development of phage therapy. Expectedly, the commercial phage products are more than those intended for plant biocontrol.

Table 2 summarizes the available data for the number of phage products and the country where they are manufactured or applied. It can be summarized by saying that approximately 20 countries worldwide produce and implement phage products. The approximate number of available phage products is 119 (according to the available sources of information) (Huang *et al*., 2022). Compared to the number of chemical preparations produced annually for the treatment of various diseases, this number is very small. It is important to note that there are a few countries that are strongly involved in phage

preparation production: the USA, Russia, Poland, Georgia, and China. Interestingly, there are many countries in Europe that produce phage preparation: Switzerland, France, Czech Republic, Germany, Norway, Ukraine, United Kingdom, Austria, The Netherlands, Poland, Georgia and Russia. The latter two are known to have a long history linked with phage application for disease treatments (Huang *et al.,* 2022). These two countries produce most of the phage products in Europe. We found seventeen phage-based products produced in Russia and 12 in Georgia. These facts are probably due to the countries' policies regarding the manufacturing and implementation of phage products into the free market.

Phage endolysins - another promising strategy against MDR bacteria

Endolysins are phage-encoded hydrolytic enzymes that can degrade several bonds in the bacterial cell wall and the murein (peptidoglycan) layer, in particular. They are produced during the late stages of the lytic phage cycle, when the bacterial cell is destroyed, and the new phage generation is released outside. Thus, the main action of phage endolysins is inside out.

The molecular structure of phage endolysins

Table 1. Studies of phages infecting phytopathogenic bacteria – a review of the recent papers (2018 – 2024).

Target bacteria	Disease	Bacteriophage	Trials	Year of publication	Reference
X. euvesicatoria pv. *euvesicatoria*	bacterial spot	BsXeu269p/3	*in vitro*	2023	Kizheva *et al*., 2023,
		BsXeu269p/3	*in planta*	2023	Shopova *et al*., 2023
		SfXv124t/3	*in vitro*	2021	Kizheva *et al*., 2021
		KΦ1	*in planta*	2018	Gasic *et al*., 2018
X. euvesicatoria pv. *perforans*	Bacterial spot	ΦXp06-02 and ΦXv3-21	*in vitro, in planta*	2018	Balogh *et al*., 2018
X. hortorum pv. *gardneri*	Bacterial spot	SfXv124t/3	*in vitro*	2021	Kizheva *et al*., 2021
X. vesicatoria	Bacterial spot	SfXv124t/3	*in vitro*	2021	Kizheva *et al*., 2021
X. oryzae pv. *oryzae*	Bacterial leaf blight	X3	*in vitro, in vivo*	2019	Ogunyemi *et al*., 2019
		vB_XooS_NR08	*in vitro, in vivo*	2023	Jain *et al*., 2023
		Xoosp13	*in vitro*	2021	Nazir *et al*., 2021
		Xoo-sp2	*in vitro, in vivo*	2018	Dong *et al.,* 2018
X. citri subsp. *citri*	Citrus canker	ΦXv3-21, ΦXaacF1, or ccΦ19-1	*in vitro, in vivo*	2018	Balogh *et al.,* 2018
		vb_XciM_LucasX	*in vitro*	2020	Marquioni et al., 2022
C. michiganensis subsp. *michiganensis*	Bacterial wilt and canker	Phage33	*in vitro*	2023	Bekircan Eski *et al*., 2023
C. michiganensis subsp. *nebraskensis*	Goss's wilt	CN8	*in vitro, in vivo*	2019	Kimmelshue *et al*., 2019
R. solanacearum	Bacterial wilt	ϕsp1	*in vitro, in vivo*	2021	Umrao *et al.,* 2021
		vRsoP-WF2, vRsoP-WM2 and vRsoP-WR2	*in vitro* *in vivo* *in vitro*	2021 2019 2023	Biosca *et al*., 2021 Alvarez *et a*l., 2019 Bertolini *et al.,* 2023
		NNP42	*in vitro*	2023	Wang *et al*., 2023
		unspecified	*in vitro, in vivo*	2019	Wang *et al*., 2019
		RsPod1EGY	*in vitro, in vivo*	2018	Elhalag *et al*., 2018

Target bacteria	Disease	Bacteriophage	Trials	Year of publication	Reference
E. amylovora	Fire blight	ΦFifi044 and ΦFifi451	*in vitro*	2022	Park *et al.*, 2022
		Hena1	*in vitro*	2020	Besarab *et al.*, 2020
		E. virus Ea35-70 and Erwinia virus Ea9-2	*in vitro*	2019	Gayder *et al.*, 2019
		pEa_SNU-ABM_12, pEa_SNUABM_47, and pEa_SNU-ABM_50	*in vitro*	2020	Kim *et al.,* 2020
		ϕEa21-4, ϕEa46-1-A1, and ϕEa35-70	*in vitro*	2020	Gayder *et al.*, 2020
		PEar1, PEar2, PEar4 and PEar6	*in vitro, in vivo*	2020	Akremi *et al.*, 2020
		vB_EamM_Y3	*in vitro*	2018	Buttimer *et al.*, 2018c
		ϕ1-ϕ28	*in vitro, ex vivo*	2024	Biosca *et al.*, 2024
P. atrosepticum	potato blackleg and soft rot	vB_PatP_CB1, vB_PatP_CB3, and vB_PatP_CB4	*in vitro, in vivo*	2018	Buttimer *et al.,* 2018b
		ϕMA1, ϕMA1A, ϕMA2, ϕMA5, ϕMA6 and ϕMA7	*in vitro, in vivo*	2020	Zaczek-Moczydłowska *et al.,* 2020
		phage Nepra, Lelidair, Nobby, Slant, Gaspode and Momine	*in vitro, in vivo*	2019	Carstens *et al.*, 2019
		vB_PatP_CB5	*in vitro*	2018	Buttimer *et al.*, 2018a
		vB_PatM_CB7	*in vitro*	2020	Buttimer *et al.*, 2020
		PP1, 2 and 7	*in vitro, in vivo*	2024	Wu *et al.,* 2024
		Jarilo	*in vitro*	2020	Pedersen *et al.,* 2020
P. carotovorum subsp. *carotovorum*	Soft rot	phage POP12, phages POP15 and POP17	*in vitro, in vivo*	2022	Kim *et al.,* 2022
		PCT27	*in vitro, in vivo*	2023	Kim *et al.*, 2023
		Wc1, Wc2, Wc6, Wc7, Wc8, Wc9, Wc10, Wc3, Wc4, Wc5, and Wc5r	*in vitro, in vivo*	2019	Muturi *et al.*, 2019
		PcaP1EGY and PcaP2EGY	*in vitro, in vivo*	2024	Elhalag *et al.,* 2024
		φPcCB7V and φPcCB251	*in vivo*	2022	Beno *et al.,* 2022

Target bacteria	Disease	Bacteriophage	Trials	Year of publication	Reference
P. syringae pv. *tomato*	Bacterial black rot	Medea1	*in vitro, in vivo*	2023	Skliros *et al*., 2023
		PH 33, PH 34	*in vitro, in vivo*	2018	Cemen *et al.,* 2018
P. syringae pv. *syringae*	Bacterial canker	MR1-MR18	*in vitro, in vivo*	2020	Rabiey *et al*., 2020
		φ6	*in vitro*	2019	Pinheiro *et al.,* 2019
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	Bacterial wilt and tan spot	Ayka	*in vitro, in planta*	2022	Tarakanov *et al.,* 2022
Burkholderia glumae	Bacterial panicle blight	NBP1-1, NBP4-7, and NBP4-8	*in vitro, in planta*	2021	Jungkhun *et al*., 2021
Dickeya solani	Soft rot and blackleg	JA10, JA11, JA13, JA29 and JA33	*in vitro*	2018	Day *et al*., 2018
		Dagda, Mysterion, Luksen, Coodle, Kamild, Ninurta	in vitro, in vivo	2018	Carstens *et al.,* 2018

is different depending on what type of bacteria they can lyse, Gram (+) or Gram (−). The first consists of two functional domains: the enzymatically active domain (EAD) in the N-terminal region and the cell wall binding domain (CBD) in the C-terminal region (Oliveira *et al*., 2013). Endolysins of phage infecting Gram (−) bacteria generally do not possess CBD (Oliveira *et al*., 2012). However, several exceptions have been reported: the endolysin KZ144, produced by phage infecting *P. aeruginosa* has CBD but at the opposite termini (Briers *et al*., 2009); the endolysin, derived from phage OBP (host bacteria *P. putida*), has two CBD (Cornelissen *et al*., 2012). However, it has been reported that generally, the CBD of endolysins, derived from phages affecting Gram (+) bacteria are host-specific, while endolysins from phages affecting Gram (−) bacteria are not (Oliveira *et al.,* 2012).

The role of the two domains in enzyme action is different. While the EAD can cleave the bonds in the murein layer (between the amino sugars and the peptide bonds), the CBD is responsible for cell wall-specific site recognition, i.e., it depends on the substrate (Chang, 2020). For example, the role of teichoic acid in the specific site recognition of endolysin PlyP35, produced by phage infecting *L. monocytogenes,* has been demonstrated (Eugster *et al*., 2011). Thus, the combined function of both domains results in higher specificity (Becker *et al*., 2015).

In the case of Gram (+) bacteria, endolysins can act not only inside out but also, destroy the cell wall from the outside in. As the major component of the Gram (+) bacteria cell wall is peptidoglycan and they lack an outer membrane, it is understandable why endolysins have such action. It is this particular feature that makes them suitable as antimicrobial agents against Gram (+) pathogens. In this regard, the application of endolysins to combat Gram (−) bacteria, is a challenge. The outer membrane in their cell walls acts like a barrier in the case of the action of the endolysins from the outside in. However, several exceptions of endolysins derived from phages affecting Gram (−) bacteria, that can destroy the cell from outside, have been reported: the endolysin LysAB2, produced by *Acinetobacteri baumanii* and the endolysin from *Salmonella* phage SPN9CC (Nazir *et al.,* 2023). Therefore, it is critical to enhance the efficiency of these endolysins with regard to their increased action against Gram (−) bacteria. Numerous approaches have been considered: 1/ the use of chelating agents (EDTA and organic acids), increasing the membrane permeability and thus facilitating the entrance of the endolysin (Chang, 2020); 2/ the use of the so-called Artilysins, modified versions of endolysins with peptides, which act like membrane-penetrating agents (Nazir *et al.,* 2023); 3/ the encapsulation of endolysins in liposomes, which act as carriers (Bai *et al.,* 2019). Moreover, as their target is that part of bacterial cells (the murein layer), which is generally put under less evolutionary pressure, the chances of mutations occurring are lesser. Thus, the target site of endolysin action will always be available, re-

Table 2. Summary data on the number phage products, produced and applied globally, based on the available information.

Target pathogen	Animal treatment		Food application		Human treatment	
	n	country	n	country	n	country
E. coli	5	USA*, Poland, Chile	3	Canada, Israel, USA	3	USA
Salmonella	8	USA, Poland, SAC**	4	USA, Canada, Israel, China	-	-
Listeria monocytogenes	1	USA	3	USA, Israel, Canada, Switzerland	-	-
Clostridium perfringens	2	USA, China	-	-		
Clostridium difficile			-	-	1	UK***
Staphylococci	1	USA	-	-	1	Georgia
Staphylococcus aureus			-	-	9	Russia, USA, China, South Korea, France, UK
Yersinia ruckeri	1	Norway	-	-		
Riemerella anatipestifer	1	China	-	-		
Weisella ceti	1	Colombia	-	-		
Campylobacter	-	-	1	USA		
Shigella	-	-	1	USA	1	USA
P. aeruginosa	-	-	-	-	7	Russia, USA, China, UK, France
Klebsiella pneumoniae	-	-	-	-	1	USA
Enterococcus	-	-	-	-	1	USA
Streptococcus	-	-	-	-	1	Russia
Gardnerella spp.	-	-	-	-	1	Germany
Fusobacterium nucleatum	-	-	-	-	1	USA
Other products with broad specter	18	South Korea, Chile, Poland, China, Canada, UK, Russia	2	USA		
Phage cocktails	-	-	-	-	40	Georgia, Russia, Czech Republic, Ukraine, UK, China, USA, South Korea
Subtotal number	38		14		67	
Total number	119					

sulting in a decreased possibility of the appearance of resistant phenotypes (Abdelrahman *et al*., 2021).

However, similar to phage-based products, the gap between the number of studies, aiming to evaluate the effectiveness of phage endolysins as antibacterial agents and the number of released and approved endolysin preparations, exists. Table 3 summarizes the selected data from the latest research on the antibacterial potential of various phage-derived endolysins for application in the human, plant, and food sectors. However, available information for manufactured and approved endolysin preparations intended for global use, is lack.

The issue of regulation of phage products in Europe

Without a doubt, Eastern and Western European nations differ significantly in terms of phage therapy‘s widespread acceptance and application. There are few countries that have successfully implemented phage therapy for human care since phages’ discovery at the beginning of the 20th century (Międzybrodzki *et al.,* 2018). These are Poland, Georgia, and Russia. Poland has been a member state of the European Union (EU) since 2004 and thus, its regulatory system is in accordance with the EU legislation. Regarding phage therapy, two European Directives are put under strict adher-

ence: the Directive of the European Parliament and of the Council (2001/20/EC) that regulates clinical trials; and the Commission Directive regulating the Good Clinical practice (2005/28/EC) (Hartmann and Hartmann-Vareilles, 2006). In general, in Poland, phage therapy is considered an experimental treatment and is administered via the so-called "compassionate use", i.e., administration of drugs that are not officially approved (Yang *et al*., 2023). The situation in Georgia and Russia is quite different, even from the Polish situation, and much more different from the rest of the European countries (Western Europe). Both countries share similarities regarding the consideration of phage products as pharmaceuticals and the free purchasing of particular phage cocktails (Yang *et al*., 2023). On the other hand, in Georgia, personalized phage preparations (PPP) are manufactured by particular pharmaceutical companies under the strict permission of the Ministry of Healthcare of the country, while in Russia those products are produced and controlled under the quality criteria given in the Russian Pharmacopoeia (Międzybrodzki *et al.*, 2018). Nevertheless, the use of PPP is prohibited in Russia (Yang *et al*., 2023). Only one company is allowed to manufacture and send phage cocktails to market (Vlassov *et al*., 2020).

In general, Western Europe‘s acceptance of phage products as an alternative to antibiotic treatment remains limited. Although certain countries attempt to use phage products to treat patients, for others this approach is still absolutely uncommon and undiscovered. One possible explanation for the current situation is that the regulatory structure in Europe, particularly in the EU, is not yet entirely ready to place phage products under concrete regulation and to define what phages and phage preparations are about the regulation.

There are still some uncertainties as to whether they are "biological medicinal products" or "advanced therapy medicinal products", although phage preparations, i.e., phage therapy have been considered medicinal products by the European Medicines Agency (EMA) since 2011 (Yang *et al*., 2023). Moreover, both types of products are under the regulation of two Commission Directives: 2001/83/EC and 2003/63/EC, respectively (Naureen *et al*., 2020). Directive 2001/83/EC of the European Parliament lays down the general rules regarding medicinal products for human use and active substances and Directive 2003/63/EC is its amended version (Directives 2001/83/EC and 2003/63/EC). Since this ambiguity has made it difficult to take effective action to produce such products, the EMA has not yet approved any phage products (Naureen *et al*., 2020). However, Directive 2001/83/EC gives the Member states of the EU the right not to apply the document in several concrete situations, listed in Articles 3.1 and 3.2., concerning medicinal products prepared in pharmacies either via magistral or official formula (Directive 2001/83/EC). In this regard, as it is written above, there are several EU countries that apply various national legislation documents and successfully regulate the production of phage-based preparations. For example, the United Kingdom's (UK) Medicine and Healthcare Products Regulatory Agency accepts phages as biological medicine and regulates the compassionate use of phage therapy, although phage therapy is not officially approved (Jones *et al*., 2023). The regulation of phage therapy in France is under the authority of the National Agency for the Safety of Medicines and Health Products, which also supervises compassionate use (Yang *et al.,* 2023). On the other hand, Belgium, unlike the UK and France, has established different rules for the application of phage therapy – through magistral preparation (Pirnay *et al*., 2018). Magistral phage products are described as products containing natural phages as active pharmaceutical ingredients (pAPI), manufactured in pharmacies/by a pharmacist, by a medical prescription for a particular personal application, i.e., PPP (Verbeken and Pirnay, 2022). It has taken many years of debate and recognition that the two aforementioned directives are not applicable in the case of application of PPP and to achieve the understanding of using the magistral route of application. In this regard, other nations can use Belgium‘s experience as a kind of model for the concept of providing therapeutic phages to people who are in need.

In the EU, the European Pharmacopoeia (Ph. Eur.) is the general guideline that establishes the rules for the production of medicinal products and sets the quality standards. Nowadays, the great goal to achieve is to implement pAPI as a separate General Monograph in the Ph. Eur. Since 2015, several requests for the implementation of pAPI in Ph. Eur. have been deposited: from the Czech Republic, France, and Belgium (Verbeken and Pirnay, 2022). As a result of the long-term discussion, in August 2021, it was proposed that a new General Chapter on phages, named 'Phage therapy active substances and medicinal products for human and veterinary use', be included in the Ph. Eur. (Verbeken and Pirnay, 2022). The draft text of this chapter has been

Table 3. Selected data of the latest research on the antibacterial potential of phage – derivate endolysins for application in human, plants and food sector

Target bacteria	Tested endolysin	Investigation	Field of application	Year of research	Reference
Streptococcus iniae	PlyGBS 90–1, PlyGBS 90–8, and ClyX-2	ClyX exhibit great lytic activity at low concentration (~15 μg/mL)	aqua cultures	2024	Deshotel *et al.*, 2024
S. epidermidis	CF-301	Exhibit remarkable antibiofilm action	Human	2022	Souche *et al.*, 2022
Enterococcus faecalis	Ply2660 in combination with antimicrobial peptide LL-37	Synergistic effect was established more effective against biofilm formation ability and against formed biofilms; The combined product inhibit the spread of *E. faecalis in vivo* (in mice models)	human	2023	Zhang *et al.*, 2023
E. faecium *E. faecalis* *S. aureus*	Ply113	Activity was observed against biofilms formed by one or two of the targeted species; Promising antibacterial results obtained in peritoneal septicemia murine model	human	2023	Wang *et al.*, 2023
S. aureus	LysGH15	The effect of fats and NaCl on enzyme activity in milk were established	Food sector	2021	Yan *et al.*, 2021
S. aureus	LysSYL	The ability to destroy mono- and mixed biofilms was demonstrated; Mouse models, infected with 10^8 cfu/mL *S. aureus* were 100% cured after treatment with 50 mg/kg of LysSYL;	Human	2024	Liu *et al.*, 2024
Salmonella Enteritidis *Salmonella* Typhimurium *Salmonella* Choleraesuis *Salmonella* Anatum *Salmonella* Pullorum *Salmonella* Indiana *L. monocytogenes* *S. aureus*	LysT144	In vitro evaluation of the lytic activity of the endolysin.	human	2020	Yang *et al.*, 2020
K. pneumoniae	LysG24 and peptide – modified LysCA	*In vitro* and *in vivo* test showed that both enzymes (native and modified) expressed effective lytic activity against the agent of pulmonary infection as the modified version showed greater lytic effect	human	2022	Lu *et al.*, 2022
C. michiganensis subsp. *michiganensis*	Endolysins from phage CMP1	*In vitro* characterization of the endolysin and evaluation of its lytic specter.	agriculture	2010	Wittmann *et al.*, 2010
C. michiganensis subsp. *nebraskensis*	Endolysin from phage CN77	*In vitro* characterization of the endolysin and evaluation of its lytic specter.	agriculture	2010	Wittmann *et al.*, 2010
P. syringae pv. *actinidiae*	LysPN09 (EDTA combined)	*In vitro* characterization of the endolysin and evaluation of its lytic specter.	agriculture	2021	Ni *et al.*, 2021
R. solanacearum	LysP2110	*In vitro* characterization of the endolysin and evaluation of its lytic specter.	agriculture	2023	Chen *et al.*, 2023

published in Pharmeuropa 35.2 and is open for public discussion until the end of June 2023. This is a crucial step in the incorporation of phages as APIs in medicinal products. The introduction of phages in the Ph. Eur. will provide makers of phage preparations with a consistent guide, resulting in the development of standardized pAPI to which stakeholders will have access, hence facilitating magistral phage manufacturing and application. However, the transformation of the General Chapter into a General Monograph may take a lot of time, probably years. Every party involved, including producers, scientists, industry, and reference laboratories, must work together to achieve this, though.

Conclusion

Phages have the potential to be used as alternatives to conventional antibacterial preparations. There is a lot of supportive information available through the vast number of scientific publications on the theme. Moreover, designer phages have been considered one of the top 10 emerging technologies, according to a flagship report summarizing the main outcomes from The World Economic Forum, held in June 2023 (World Economic Forum, 2023, accessed on April 2024). Thus, it is critical to promote phage research, particularly in nations with little or no experience with phage studies as therapeutic agents.

However, there is still one critical issue concerning the general understanding of the use of bacteriophages as antibacterial agents that needs further clarification among the public. After the COVID-19 pandemic, people are very sensitive about viruses. Ordinary people associate the word „virus“ with something that will infect them and, in some cases, result in a lethal end. Therefore, it is not an effective strategy to tell people that phages are “viruses”, because they are probably unlikely to understand the distinction between human viruses and bacterial viruses; for them, a virus is a virus. So, this approach probably will not result in building public confidence in bacteriophages. If we try to explain to people that phage therapy is an approach based on “natural bacterial killers” rather than “bacterial viruses”, it will result in greater understanding and acceptance among the common population (McCammon *et al*., 2023). Therefore, the potential of phages as antibacterial agents, as well as their usage in a variety of fields (e.g., human and animal healthcare, agriculture, aquaculture, and the food sector), must be communicated carefully but sustainably.

Acknowledgments

Phage studies in Bulgaria are financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No BG-RRP-2.004-0008.

References

Abdelrahman, F., M. Easwaran, O. I. Daramola, S. Ragab, S. Lynch, T. J. Oduselu, F. M. Khan, A. Ayobami, F. Adnan, E. Torrents, S. Sanmukh, A. El-Shibiny (2021). Phage-encoded endolysins. *Antibiotics* **10**: 124. doi: 10.3390/antibiotics10020124.

Ahiwale, S., P. Koparde, P. Deore, V. Gunale, B. P. Kapadnis (2012). Bacteriophage based technology for disinfection of different water systems. In: Satyanarayana, T., B. Johri (Eds.) Microorganisms in Environmental Management. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-2229-3_14.

Akbaba, M., H. Ozaktan (2021). Evaluation of bacteriophages in the biocontrol of *Pseudomonas syringae* pv. *syringae* isolated from cankers on sweet cherry (*Prunus avium L*.) in Turkey. *Egypt. J. Biol. Pest. Control* **31**: 35. doi:10.1186/s41938-021-00385-7.

Akremi, I., D. Holtappels, W. Brabra, M. Jlidi, A. H. Ibrahim, M. B. Ali, K. Fortuna, M. Ahmed, B. V. Meerbeek, A. Rhouma, R. Lavigne, M. B. Ali, J. Wagemans (2020). First report of filamentous phages isolated from Tunisian orchards to control *Erwinia amylovora*. *Microorganism*s **8**: 1762. doi: 10.3390/microorganisms8111762.

Ali, S. F., S. H. Teh, H. H. Yang, Y. C. Tsai, H. J. Chao, S. S. Peng, S. C. Chen, L. C. Lin, N. T. Lin (2024). Therapeutic potential of a novel lytic phage, vB_EclM_ECLFM1, against carbapenem-resistant *Enterobacter cloacae*. *Int. J. Mol. Sci*. **25**: 854. https://doi.org/10.3390/ijms25020854.

Alvarez, B., M. M. Lopez, E. G. Biosca (2019). Biocontrol of the major plant pathogen *Ralstonia solanacearum* in irrigation water and host plants by novel waterborne lytic bacteriophages. *Front. Microbiol*. **10**: 1-17. doi:10.3389/fmicb.2019.02813.

Bai, J., E. Yang, P. S. Chang, S. Ryu (2019). Preparation and characterization of endolysin-containing liposomes and evaluation of their antimicrobial activities against gram-negative bacteria. *Enzym. Microb. Technol*. **128**: 40–48. doi: 10.1016/j.enzmictec.2019.05.006.

Balogh, B., N. T. T. Nga, J. B. Jones (2018). Relative level of bacteriophage multiplication in vitro or in phyllosphere may not predict in planta efficacy for controlling bacterial leaf spot on tomato caused by *Xanthomonas perforans*. *Front. Microbiol.* **9**: 2176. doi: 10.3389/fmicb.2018.02176.

Becker, S. C., S. Swift, O. Korobova, N. Schischkova, P. Kopylov, D. M. Donovan, I. Abaev (2015). Lytic activity of the staphylolytic Twort phage endolysin CHAP domain is enhanced by the SH3b cell wall binding domain. *FEMS Microbiol. Lett*. **362**: 1–8. doi: 10.1093/femsle/fnu019.

Bekircan Eski, D., C. Darcan (2023). Isolation *of Clavibacter michiganensis* subsp. *michiganenesis*-specific bacteriophages from tomato fields in Turkey and their biocontrol potential. *Egypt. J. Biol. Pest Control.* **33**: 71. doi: 10.1186/s41938-023-00717-9.

Beňo, F., I. Horsáková, M. Kmoch, K. Petrzik, G. Krátká, R.

Ševčík (2022). Bacteriophages as a strategy to protect potato tubers against *Dickeya dianthicola* and *Pectobacterium carotovorum* Soft Rot. *Microorganisms* **10**: 2369. doi: 10.3390/microorganisms10122369.

Bertolini, E., A. Figàs-Segura, B. Álvarez, E. G. Biosca (2023). Development of TaqMan real-time PCR protocols for simultaneous detection and quantification of the bacterial pathogen *Ralstonia solanacearum* and their specific lytic bacteriophages. *Viruses* **15**: 841. doi: 10.3390/v15040841.

Besarab, N. V., A. E. Akhremchuk, M. A. Zlatohurska, L. V. Romaniuk, L. N. Valentovich, F. I. Tovkach, A. L. Lagonenko, A. N. Evtushenkov (2020). Isolation and characterization of Hena1 - a novel *Erwinia amylovora* bacteriophage. *FEMS Microbiol. Lett*. **367**: fnaa070. doi:10.1093/femsle/fnaa070.

Biosca, E. G., J. F. Català-Senent, A. Figàs-Segura, E. Bertolini, M. M. López, B. Álvarez (2021). Genomic analysis of the first European bacteriophages with depolymerase activity and biocontrol efficacy against the phytopathogen *Ralstonia solanacearum*. *Viruses* **13**: 2539. doi: 10.3390/v13122539.

Biosca, E. G., R. Delgado Santander, F. Morán, A. Figàs-Segura, R. Vázquez, J. F. Català-Senent, B. Álvarez (2024). First European *Erwinia amylovora* lytic bacteriophage cocktails effective in the host: characterization and prospects for fire blight biocontrol. *Biology* **13**: 176. doi: 10.3390/biology13030176.

Breitbart, M., F. Rohwer (2005). Here a virus, there a virus, everywhere the same virus? *Trends Microbiol*. **13**: 278-284. doi: 10.1016/j.tim.2005.04.003.

Briers, Y., M. Schmelcher, M. J. Loessner, J. Hendrix, Y. Engelborghs, G. Volckaert, R. Lavigne (2009). The high-affinity peptidoglycan binding domain of *Pseudomonas* phage endolysin KZ144. *Biochem. Biophys. Res. Commun*. **383**: 187–191. doi:10.1016/j.bbrc.2009.03.161.

Buttimer, C., A. Lucid, H. Neve, C. M. A. P. Franz, J. O'Mahony, D. Turner, R. Lavigne, A. Coffey (2018a). *Pectobacterium atrosepticum* phage vB_PatP_CB5: a member of the proposed genus '*Phimunavirus*'. Viruses **10**: 394. doi:10.3390/v10080394.

Buttimer, C., C. Lynch, H. Hendrix, H. Neve, J. P. Noben, R. Lavigne, A. Coffey (2020). Isolation and characterization of *Pectobacterium* Phage vB_PatM_CB7: new insights into the genus *Certrevirus*. Antibiotics **9**: 352. doi:10.3390/antibiotics9060352.

Buttimer, C., H. Hendrix, A. Lucid, H. Neve, J. P Noben, C. Franz, J. O'Mahony, R. Lavigne, A. Coffey (2018b). Novel N4-Like bacteriophages of *Pectobacterium atrosepticum*. *Pharmaceuticals* **11**: 45. doi:10.3390/ph11020045.

Buttimer, C., O. McAuliffe, R. P. Ross, C. Hill, J. O'Mahony, A. Coffey (2017). Bacteriophages and bacterial plant diseases. *Front. Microbiol*. **8**: 212667. doi:10.3389/fmicb.2017.00034.

Buttimer, C., Y. Born, A. Lucid, M. J. Loessner, L. Fieseler, A. Coffey (2018c). *Erwinia amylovora* phage vB_EamM_Y3 represents another lineage of hairy *Myoviridae*. *Res. Microbiol*. **169**: 505-514. doi:10.1016/j.resmic.2018.04.006.

Capparelli, R., N. Nocerino, M. Iannaccone, D. Ercolini, M. Parlato, M. Chiara, D. Iannelli (2010). Bacteriophage therapy of *Salmonella enterica*: a fresh appraisal of bacteriophage therapy. *J. Infect. Dis*. **201**: 52–61. doi: 10.1086/648478.

Carstens, A. B., A. M. Djurhuus, W. Kot, L. H. Hansen (2019). A novel six-phage cocktail reduces *Pectobacterium atrosepticum* soft rot infection in potato tubers under simulated storage conditions. *FEMS Microbiol Lett*. **366**: 1-8. doi:10.1093/femsle/fnz101.

Carstens, A. B., A. M. Djurhuus, W. Kot, D. Jacobs-Sera, G. F. Hatfull, L. H. Hansen (2018). Unlocking the potential of 46 new bacteriophages for biocontrol of *Dickeya solani*. *Viruses* **10**: 1-12. doi:10.3390/v10110621.

Cemen, A., H. Saygili, S. Horuz, Y. Aysan. (2018). Potential of bacteriophages to control bacterial speck of tomato (*Pseudomonas syringae* pv. *tomato*). *Fresenius Environ. Bull*. **27**: 9366-9373.

Chang, Y. (2020). Bacteriophage-derived endolysins applied as potent biocontrol agents to enhance food safety. *Microorganisms* **8**: 724. https://doi.org/10.3390/microorganisms8050724.

Chattopadhyay, D., S. Chattopadhyay, W. G. Lyon, J. T. Wilson (2002). Effect of surfactants on the survival and sorption of viruses. *Environ. Sci. Technol*. **36**: 4017–4024. doi: 10.1021/es0114097.

Chen, K., Y. Guan, R. Hu, X. Cui, Q. Liu (2023). Characterization of the LysP2110-HolP2110 lysis system in *Ralstonia solanacearum* phage P2110. *Int. J. Mol. Sci.* **24**: 10375. doi:10.3390/ijms241210375.

Chibeu, A., L. Agius, A. Gao, P. M. Sabour, A. M. Kropinski, S. Balamurugan (2013). Efficacy of bacteriophage LISTEX™P100 combined with chemical antimicrobials in reducing *Listeria monocytogenes* in cooked turkey and roast beef. *Int. J. Food Microbiol*. **167**: 208–214. doi:10.1016/j.ijfoodmicro.2013.08.018.

Cieślik, M., M. Harhala, F. Orwat, K. Dąbrowska, A. Górski, E. Jończyk-Matysiak (2022). Two newly isolated *Enterobacter*-specific bacteriophages: biological properties and stability studies. *Viruses* **14**: 1518. doi: 10.3390/v14071518.

Coons, G., J. Kotila (1925). The transmissible lytic principle (bacteriophage) in relationto plant pathogens. *Phytopathology* **15**: 357–370.

Cornelissen, A., S. C. Hardies, O. V. Shaburova, V. N. Krylov, W. Mattheus, A. M. Kropinski, R. Lavigne (2012). Complete genome sequence of the giant virus OBP and comparative genome analysis of the diverse KZ-related phages. *J. Virol*. **86**: 1844–1852. DOI:10.1128/JVI.06330-11.

d'Herelle, F. (1917). Sur un microbe invisible antagoniste des Bacillies dysentériques. *C. R. Acad. Sci*. **165**: 373–375.

Day, A., J. Ahn, G. P. C. Salmond (2018). Jumbo bacteriophages are represented within an increasing diversity of environmental viruses infecting the emerging phytopathogen, *Dickeya solani*. *Front. Microbiol*. **9**: 2169. doi: 10.3389/fmicb.2018.02169.

Dedrick, R., C. Guerrero-Bustamante, R. A. Garlena, D. A. Russell, K. Ford, K. Harris, K. C. Gilmour, J. Soothill, D. Jacobs-Sera, R. T. Schooley, G. F. Hatfull, H. Spencer (2019). Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. *Nat. Med.* **25**: 730–733. doi: 10.1038/s41591-019-0437-z.

Delitheos, A., E. Tiligada, A. Yannitsaros, I. Bazos (1997). Antiphage activity in extracts of plants growing in Greece. *Phytomedicine* **4**: 117–124. doi: 10.1016/ S0944-7113(97)80055-4.

Deshotel, M. B., U. M. Dave, B. Farmer, D. Kemboi, D. C. Nelson (2024). Bacteriophage endolysin treatment for systemic infection of *Streptococcus iniae* in hybrid striped bass. *Fish Shellfish Immunol*. **145**: 109296. doi:10.1016/j.fsi.2023.109296.

Diana, G., R. R. Lorena, F. Lucía, M. Beatriz, R. Ana, G. Pilar (2017). Applicability of commercial phage-based products against *Listeria monocytogenes* for improvement of food safety in Spanish dry-cured ham and food contact surfaces. *Food Control***.** **73**: 1474–1482. https://doi.org/10.1016/j.foodcont.2016.11.007.

Directive 2001/83/EC of the European Parliament and the Council on the Community code relating to medicinal products for human use (2001). Official Journal of the European Communities.

Directive 2003/63/EC of 25 June 2003 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use (Text with EEA relevance). Official Journal of the European Communities.

Dong, Z., S. Xing, J. Liu, X. Tang, L. Ruan, M. Sun, Y. Tong, D. Peng (2018). Isolation and characterization of a novel phage Xoo-sp2 that infects *Xanthomonas oryzae pv. oryzae*. *J. Gen. Virol.* **99**: 1453-1462. doi:10.1099/jgv.0.001133.

Durand, G. A., D. Raoult, G. Dubourg (2019). Antibiotic discovery: history, methods and perspectives. *Int. J. Antimicrob*. **53**: 371-382. doi: 10.1016/j.ijantimicag.2018.11.010.

EcoPhage Ltd. https://ecophage.com/

Elhalag, K., M. Nasr-Eldin, A. Hussien, A. Ahmad (1018). Potential use of soilborne lytic *Podoviridae* phage as a biocontrol agent against *Ralstonia solanacearum*. *J. Basic Microbiol*. **58**: 658-669. doi:10.1002/jobm.201800039.

Elhalag, K., M. A. Mohamed, Q. Huang, A. M. Rabab, A. Ali Ahmad (2024). Lytic phages isolated from Egypt for biocontrol of potato soft rot caused by *Pectobacterium carotovorum*. *Biol. Control* **189**: 105444. https://doi.org/10.1016/j.biocontrol.2024.105444.

Erskine, J. M. (1973). Characteristics of *Erwinia amylovora* bacteriophage and its possible role in the epidemiology of fire blight. *Can. J. Microbiol*. **19**: 837–845. doi: 10.1139/m73-134.

Eugster, M. R., M. C. Haug, S. G. Huwiler, M. J. Loessner (2011). The cell wall binding domain of *Listeria* bacteriophage endolysin PlyP35 recognizes terminal GlcNAc residues in cell wall teichoic acid. *Mol. Microbiol*. **81**: 1419–1432. doi:10.1111/j.1365-2958.2011.07774.x.

Farrar, M. D., K. M. Howson, R. A. Bojar, D. West, J. C. Towler, J. Parry, K. Pelton, K. T. Holland (2007). Genome sequence and analysis of a *Propionibacterium acnes* bacteriophage. *J. Bacteriol*. **189**: 4161–4167. doi:10.1128/JB.00106-07.

Feiner, R., T. Argov, L. Rabinovich, N. Sigal, I. Borovok, A. A. Herskovits (2015). A new perspective on lysogeny: prophages as active regulatory switches of bacteria. *Nat. Rev. Microbiol.* **13**: 641-650. doi: 10.1038/nrmicro3527.

Fleming, A. Nobel Lecture. NobelPrize.org. Nobel Prize Outreach AB 2024. Thu. 4 Apr 2024. https://www.nobelprize.org/prizes/medicine/1945/fleming/lecture/.

García, P., R. Tabla, H. Anany, R. Bastias, L. Brøndsted, S. Casado, P. Cifuentes, J. Deaton, T. G. Denes, M. A. Islam, R. Lavigne, A. I. Moreno-Switt, N. Nakayama, C. M. Madero, A. Sulakvelidze, A. M. Svircev, J. Wagemans, E. G. Biosca, D. Rivera (2023). ECOPHAGE: combating antimicrobial resistance using bacteriophages for eco-sustainable agriculture and food systems. *Viruses* **15**: 2224. doi: 10.3390/v15112224.

Gašić, K., N. Kuzmanović, M. Ivanović, A. Prokić, M. Šević, A. Obradović (2018). Complete genome of the *Xanthomonas euvesicatoria* specific bacteriophage KΦ1, its survival and potential in control of pepper bacterial spot. *Front. Microbiol*. **9**: 2021. doi: 10.3389/fmicb.2018.02021.

Gayder, S., M. Parcey, A. J. Castle, A. M. Svircev (2019). Host range of bacteriophages against a world-wide collection of *Erwinia amylovora* determined using a quantitative PCR assay. *Viruses* **11**: 910. doi: 10.3390/v11100910.

Gayder, S., M. Parcey, D. Nesbitt, A. J. Castle, A. M. Svircev (2020). Population dynamics between *Erwinia amylovora*, *Pantoea agglomerans* and bacteriophages: exploiting synergy and competition to improve phage cocktail efficacy. *Microorganisms* **8**: 1449. doi: 10.3390/microorganisms8091449.

Gordillo Altamirano, F. L., J. J. Barr (2019). Phage therapy in the postantibiotic era. *Clin. Microbiol. Rev*. **32**: e00066-18. doi: 10.1128/CMR.00066-18.

Grant, A., S. Parveen, J. Schwarz, F. Hashem, B. Vimini (2017). Reduction of *Salmonella* in ground chicken using a bacteriophage. *Poult. Sci*. **96**: 2845–2852. doi: 10.3382/ps/pex062. PMID: 28371846.

Gupta, R., Y. Prasad (2010). Efficacy of polyvalent bacteriophage P-27/HP to control multidrug resistant *Staphylococcus aureus* associated with human infections. *Curr. Microbiol.* **62**: 255–260. doi: 10.1007/s00284-010-9699-x.

Hartmann, M., F. Hartmann-Vareilles (2006). The clinical trials directive: how is it affecting Europe's noncommercial research. *PLoS Clin. Trials* **1**: e13. doi: 10.1371/journal.pctr.0010013.

Hatfull, G. F., R. M. Dedrick, R. T. Schooley (2022). Phage therapy for antibiotic-resistant bacterial infections. *Annu. Rev. Med.* **73**: 197-211. doi:10.1146/annurev-med-080219-122208.

Holtappels, D., K. Fortuna, R. Lavigne, J. Wagemans (2021). The future of phage biocontrol in integrated plant protection for sustainable crop production. *Curr. Opin. Biotechnol*. **68**: 60-71. doi: 10.1016/j.copbio.2020.08.016.

Holtappels, D., K. J. Fortuna, L. Moons, N. Broeckaert, L. E. Bäcker, S. Venneman, S. Rombouts, L. Lippens, S. Baeyen, S. Pollet, J. P. Noben, F. Oechslin, M. Vallino, A. Aertsen, M. Maes, J. Van Vaerenbergh, R. Lavigne, J. Wagemans (2022). The potential of bacteriophages to control *Xanthomonas campestris* pv. *campestris* at different stages of disease development. *Microb. Biotechnol.* **15**: 1762-1782. doi: 10.1111/1751-7915.14004.

Huang, Y., W. Wang, Z. Zhang, Y. Gu, A. Huang, J. Wang, H. Hao (2022). Phage products for fighting antimicrobial resistance. *Microorganisms* **10**: 1324. doi: 10.3390/ microorganisms10071324.

Hyman, P. (2019). Phages for phage therapy: isolation, characterization, and host range breadth. *Pharmaceuticals* **12**: 35. doi: 10.3390/ph12010035

IACG (Interagency Coordination group on Antimicrobial Resistance) (2019). No Time to Wait: Securing the Future From Drug-Resistant Infections. World Health Organization. Report to the secretary-general of the Unit-

ed Nations. Available online at: https://www.who.int/antimicrobial-resistance/interagency-coordination-group/IACG_final_report_EN.pdf?ua=1%E2%80%9D.

Iriarte, F. B., B. Balogh, M. T. Momol, L. M. Smith, M. Wilson, J. B. Jones (2007). Factors affecting survival of bacteriophage on tomato leaf surfaces. *Appl. Environ. Microbiol*. **73**: 1704–1711. doi: 10.1128/AEM.02118-06.

Jain, L., V. Kumar, S. K. Jain, P. Kaushal, P. K. Ghosh (2023). Isolation of bacteriophages infecting *Xanthomonas oryzae* pv. *oryzae* and genomic characterization of novel phage vB_XooS_NR08 for biocontrol of bacterial leaf blight of rice. *Front. Microbiol*. **14**: 1084025. doi: 10.3389/fmicb.2023.1084025.

Jakhetia, R., K. A. Talukder, N. K. Verma (2013). Isolation, characterization and comparative genomics of bacteriophage SfIV: a novel serotype converting phage from *Shigella flexneri*. *BMC. Genom* **14**: 677. doi:10.1186/1471-2164-14-677.

Jensen, E. C., H. S. Schrader, B. Rieland, T. L. Thompson, K. W. Lee, K. W. Nickerson, T. A. Kokjohn (1998). Prevalence of broad-host-range lytic bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol*. **64**: 575–580. doi: 10.1128/AEM.64.2.575-580.1998.

Ji, M., Z. Liu, K. Sun, Z. Li, X. Fan, Q. Li (2021). Bacteriophages in water pollution control: advantages and limitations. *Front. Environ. Sci. Eng*. **15**: 84. doi: 10.1007/s11783-020-1378-y.

Jones, J. D., C. Trippett, M. Suleman, M. R. J. Clokie, J. R. Clark (2023). The future of clinical phage therapy in the United Kingdom. *Viruses* **15**: 721. doi: 10.3390/v15030721.

Jungkhun, N., A. R. G. Farias, I. Barphagha, S. Patarapuwadol, J. H. Ham (2021). Isolation and characterization of bacteriophages infecting *Burkholderia glumae*, the major causal agent of bacterial panicle blight in rice. *Plant Dis.* **105**: 2551-2559. doi:10.1094/PDIS-08-20-1711-RE.

Karumidze, N., I. Kusradze, S. Rigvava, M. Goderdzishvili, K. Rajakumar, Z. Alavidze (2013). Isolation and characterisation of lytic bacteriophages of *Klebsiella pneumoniae* and *Klebsiella oxytoca*. *Curr. Microbiol*. **66**: 251–258. doi:10.1007/s00284-012-0264-7.

Kim, G., J. H. Kim, M. Kim (2023). Potential of bacteriophage PCT27 to reduce the use of agrochemicals to control *Pectobacterium carotovorum* subsp. *carotovorum* in Chinese cabbage (*Brassica pekinensis*). *Food Control***.** **154**: 109985. doi: 10.1016/j.foodcont.2023.109985.

Kim, S. G., S. B. Lee, S. S. Giri, H. J. Kim, S. W. Kim, J. Kwon, J. Park, E. Roh, S. C. Park (2020). Characterization of novel *Erwinia amylovora* jumbo bacteriophages from *Eneladusvirus* genus. *Viruses* **12**: 1373. doi: 10.3390/v12121373.

Kim, H., M. Kim, S. Jee, S. Heu, S. Ryu (2022). Development of a bacteriophage cocktail against *Pectobacterium carotovorum* subsp. *carotovorum* and its effects on *Pectobacterium* virulence. *Appl. Environ. Microbiol*. **88**: e00761-22. doi: 10.1128/aem.00761-22.

Kimmelshue, C., A. S. Goggi, R. Cademartiri (2019). The use of biological seed coatings based on bacteriophages and polymers against *Clavibacter michiganensis* subsp. *Nebraskensis* in maize seeds. *Sci. Rep*. **9**: 17950. doi: 10.1038/s41598-019-54068-3.

Kizheva, Y., Z. Urshev, M. Dimitrova, N. Bogatzevska, P. Moncheva, P. Hristova (2023). Phenotypic and genotypic characterization of newly isolated *Xanthomonas euvesicatoria*-specific bacteriophages and evaluation of their biocontrol potential. *Plants* **12**: 947. doi: 10.3390/plants12040947.

Kizheva,Y., M. Eftimova, R. Rangelov, N. Micheva, Z. Urshev, I. Rasheva, P. Hristova (2021). Broad host range bacteriophages found in rhizosphere soil of a healthy tomato plant in Bulgaria. *Heliyon* **7**: e07084, doi:10.1016/j.heliyon.2021.e07084.

Kotila, J., G. Coons (1925). Investigations on the blackleg disease of potato. *Michigan Agric. Exp. Stn. Tech. Bull.* **67**: 3–29.

Levin, B. R., J. J. Bull (2004). Population and evolutionary dynamics of phage therapy. *Nat. Rev. Microbiol.* **2**: 166–173. doi: 10.1038/nrmicro822.

Li, E., X. Wei, Y. Ma, Z. Yin, H. Li, W. Lin, X. Wang, C. Li, Z. Shen, R. Zhao, H. Yang, A. Jiang, W. Yang, J. Yuan, X. Zhao (2016). Isolation and characterization of a bacteriophage phiEap-2 infecting multidrug resistant *Enterobacter aerogenes*. *Sci. Rep*. **6**: 28338. doi:10.1038/srep28338.

Liu, H., X. Wei, Z. Wang, X. Huang, M. Li, Z. Hu, K. Zhang, Q. Hu, H. Peng, W. Shang, Y. Yang, Y. Wang, S. Lu, X. Rao (2024). LysSYL: a broad-spectrum phage endolysin targeting *Staphylococcus* species and eradicating *S. aureus* biofilms. *Microb. Cell Fact*. **23**: 89. doi:10.1186/s12934-024-02359-4.

Liu, J., R. Yan, Q. Zhong, S. Ngo, N. J. Bangayan, L. Nguyen, T. Lui, M. Liu, M. C. Erfe, N. Craft, S. Tomida, L. Huiying (2015). The diversity and host interactions of *Propionibacterium acnes* bacteriophages on human skin. *ISME J.* **9**: 2078–2093. doi:10.1038/ismej.2015.47.

Loc-Carrillo, C., S. T. Abedon (2011). Pros and cons of phage therapy. *Bacteriophage* **1**: 111–114. doi: 10.4161/bact.1.2.14590.

Lu, B., X. Yao, G. Han, Z. Luo, J. Zhang, K. Yong, Y. Wang, Y. Luo, Z. Yang, M. Ren, S. Cao (2022). Isolation of *Klebsiella pneumoniae* phage vB_KpnS_MK54 and pathological assessment of endolysin in the treatment of pneumonia mice model. *Front. Microbiol.* **13**: 854908. doi: 10.3389/fmicb.2022.854908.

Magiorakos, A. P., A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber, D. L. Monnet (2012). Multidrug-resistant, extensively drug-resistant and pandrugresistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect*. **18**: 268–281. doi:10.1111/j.1469-0691.2011.03570.x.

Mallmann, W., C. Hemstreet (1924). Isolation of an inhibitory substance from plants. *Agric. Res.* **28**: 599–602.

Marquioni, V., F. P. N. Rossi, D. C. Mendonça, L. F. Martins, F. Behlau, J. C. Setubal, A. M. da Silva, M. T. M. Novo-Mansur (2022). Isolation and characterization of vB_XciM_LucasX, a new jumbo phage that infects *Xanthomonas citri* and *Xanthomonas fuscans*. *PLoS ONE.* **17**: e0266891. doi:10.1371/journal.pone.0266891.

McCammon, S., K. Makarovs, S. Banducci, V. Gold (2023). Phage therapy and the public: increasing awareness essential to widespread use. *PLoS ONE* **18**: e0285824.

doi:10.1371/journal.pone.0285824.

Międzybrodzki, R., N. Hoyle, F. Zhvaniya, M. Łusiak-Szelachowska, B. Weber-Dąbrowska, M. Łobocka, J. Borysowski, Z. Alavidze, E. Kutter, A. Górski, L. Gogokhia (2018). Current updates from the long-standing phage research centers in Georgia, Poland, and Russia. In: Harper, D., S. Abedon, B. Burrowes, M. McConville (Eds.), Bacteriophages. Springer, Cham, pp. 1-31. https://doi.org/10.1007/978-3-319-40598-8_31-1.

Muturi, P., J. Yu, A. N. Maina, S. Kariuki, F. B. Mwaura, H. Wei (2019). Bacteriophages isolated in China for the control of *Pectobacterium carotovorum* causing potato soft rot in Kenya. *Virol. Sin*. **34**: 287–294. doi:10.1007/s12250-019-00091-7.

Naureen, Z., D. Malacarne, K. Anpilogov, A. Dautaj, G. Camilleri, S. Cecchin, S. Bressan, A. Casadei, E. Albion, E. Sorrentino, T. Beccari, M. Dundar, M. Bertelli (2020). Comparison between American and European legislation in the therapeutical and alimentary bacteriophage usage. *Acta Biomed.* **91**: e2020023. doi: 10.23750/abm.v91i13-S.10815.

Nazir, A., X. Xu, Y. Liu, Y. Chen (2023). Phage endolysins: advances in the world of food safety. *Cells* **12**: 2169. doi:10.3390/cells12172169.

Nazir, A., Z. Dong, J. Liu, R. A. Tahir, M. Rasheed, H. Qing, D. Peng, Y. Tong (2021). Genomic analysis of bacteriophage Xoo-sp13 infecting *Xanthomonas oryzae* pv. *oryzae*. *Arch. Virol*. **166**: 1263–1265. doi:10.1007/s00705-021-04985-4.

Ni, P., L. Wang, B. Deng, S. Jiu, C. Ma, C. Zhang, A. Almeida, D. Wang, W. Xu, S. Wang (2021). Characterization of a lytic bacteriophage against *Pseudomonas syringae* pv. *Actinidiae* and its endolysin. *Viruses* **13**: 631. doi:10.3390/v13040631.

Ogunyemi, S. O., J. Chen, M. Zhang, L. Wang, M. M. I. Masum, C. Yan, Q. An, B. Li, J. Chen (2019). Identification and characterization of five new OP2-related *Myoviridae* bacteriophages infecting different strains of *Xanthomonas oryzae* pv. *oryzae*. *J. Plant. Pathol*. **101**: 263-273. doi:10.1007/s42161-018-0188-6.

Oliveira, H., J. Azeredo, R. Lavigne, L. D. Kluskens (2012). Bacteriophage endolysins as a response to emerging foodborne pathogens. *Trends Food Sci. Technol*. **28**: 103–115. doi:10.1016/j.tifs.2012.06.016.

Oliveira, H., L. D. Melo, S. B. Santos, F. L. Nóbrega, E. C. Ferreira, N. Cerca, J. Azeredo, L. D. Kluskens (2013). Molecular aspects and comparative genomics of bacteriophage endolysins. *J. Virol*. **87**: 4558–4570. doi:10.1128/JVI.03277-12.

Pantucek, R., A. Rosypalova, J. Doskar, J. Kailerova, V. Ruzicková, P. Borecka, S. Snopková, R. Horváth, F. Götz, S. Rosypal (1998). The polyvalent staphylococcal phage φ812: its host-range mutants and related phages. *Virology* **246**: 241–252. doi: 10.1006/viro. 1998.9203.

Paolozzi, L., P. Ghelardini (2006). The bacteriophage Mu. In: Calendar R., S. T. Abedon (Eds.). The Bacteriophages. Oxford University Press, USA, pp. 469–496.

Park, J., B. Kim, S. Song, Y. W. Lee, E. Roh (2022). Isolation of nine bacteriophages shown effective against *Erwinia amylovora* in Korea. *Plant Pathol. J.* **38**: 248-253. doi: 10.5423/PPJ.NT.11.2021.0172.

Pedersen, J. S., A. B. Carstens, A. M. Djurhuus, W. Kot, H. Neve, L. H. Hansen (2020). Pectobacterium phage Jarilo displays broad host range and represents a novel genus of bacteriophages within the family *Autographiviridae*. *Phage* **1**: 237-244. doi:10.1089/phage.2020.0037.

Pinheiro, L. A. M., C. Pereira, C. Frazão, V. M. Balcão, A. Almeida (2019). Efficiency of phage φ6 for biocontrol of *Pseudomonas syringae* pv. *syringae*: an in vitro preliminary study. *Microorganisms* **7**: 286. doi:10.3390/microorganisms7090286.

Pirnay, J. P., G. Verbeken (2023). Magistral phage preparations: is this the model for everyone? *Clin. Infect. Dis*. **77**: 360–S369. doi:10.1093/cid/ciad481.

Pirnay, J. P., G. Verbeken, P. J. Ceyssens, I. Huys, D. De Vos, C. Ameloot, A. Fauconnier (2018). The Magistral phage. *Viruses* **10**: 64. doi: 10.3390/v10020064.

Rabiey, M., S. R. Roy, D. Holtappels, L. Franceschetti, B. J. Quilty, R. Creeth, G. W. Sundin, J. Wagemans, R. Lavigne, R. W. Jackson (2020). Phage biocontrol to combat *Pseudomonas syringae* pathogens causing disease in cherry. *Microb. Biotechnol*. **13**: 1428–1445. doi:10.1111/1751-7915.13585.

Rasool, M. H., R. Yousaf, A. B. Siddique, M. Saqalein, M. Khurshid (2016). Isolation, characterization, and antibacterial activity of bacteriophages against methicillin-resistant *Staphylococcus aureus* in Pakistan. Jundishapur. *J. Microbiol*. **9**: e36135. doi:10.5812/jjm.36135.

Ronda, C., R. Lopez, E. Garcia (1981). Isolation and characterization of a new bacteriophage, Cp-1, infecting *Streptococcus pneumoniae*. *J. Virol.* **40**: 551–559. doi:10.1128/JVI.40.2.551-559.1981.

Ross, A., S. Ward, P. Hyman (2016). More is better: selecting for broad host range bacteriophages. *Front. Microbiol*. **7**: 1352. doi:10.3389/fmicb.2016.01352.

São-José, C., A. R. Costa, L. D. R. Melo (2022). Editorial: bacteriophages and their lytic enzymes as alternative antibacterial therapies in the age of antibiotic resistance. *Front. Microbiol*. **13**: 884176. doi: 10.3389/fmicb.2022.884176.

Shopova, E., L. Brankova, S. Ivanov, Z. Urshev, L. Dimitrova, M. Dimitrova, P. Hristova, Y. Kizheva (2023). *Xanthomonas euvesicatoria*-specific bacteriophage BsXeu269p/3 reduces the spread of bacterial spot disease in pepper plants. *Plants* **12**: 3348. doi: 10.3390/plants12193348.

Shrivastava, S. R., P. S. Shrivastava, J. Ramasamy (2018). Responding to the challenge of antibiotic resistance: World Health Organization. *J. Res. Med. Sci*. **23**: 21. doi:10.4103/1735-1995.228593.

Skliros, D., P. Papazoglou, D. Gkizi, E. Paraskevopoulou, P. Katharios, D. E. Goumas, S. Tjamos, E. Flemetakis (2023). In planta interactions of a novel bacteriophage against *Pseudomonas syringae pv. tomato*. *Appl. Microbiol. Biotechnol*. **107**: 3801–3815. doi:10.1007/s00253-023-12493-5.

Souche, A., C. Kolenda, J. Teoli, R. Schuch, T. Ferry, F. Laurent, J. Josse (2022). Activity of *Exebacase* (CF-301) against biofilms formed by *Staphylococcus epidermidis* strains isolated from prosthetic joint infections. *Antimicrob. Agents Chemother*. **66**: e0058822. doi: 10.1128/aac.00588-22.

Sulakvelidze, A., Z. Alavidze, J. G. Morris (2001). Bacteriophage therapy. *Antimicrob.Agents Chemother*. **45**: 649–659. doi: 10.1128/AAC.45.3.649-659. 2001.

Svircev, A., D. Roach, A. Castle (2018). Framing the future

with bacteriophages in agriculture. *Viruses* **10**: 218. doi: 10.3390/v10050218.

Tarakanov, R. I., A. A. Lukianova, P. V. Evseev, R. I. Pilik, A. D. Tokmakova, E. E. Kulikov, S. V. Toshchakov, A. N. Ignatov, F. S. U. Dzhalilov, K. A. Miroshnikov (2022). Ayka, a novel *Curtobacterium* bacteriophage, provides protection against soybean bacterial wilt and tan spot. *Int. J. Mol. Sci*.**23**: 10913. doi:10.3390/ijms231810913.

Tay, A. (2023). How building a phage directory can mean life or death for patients. *Nature* doi: 10.1038/d41586-023-02209-0.

Thomas, R. (1935). A bacteriophage in relation to Stewart's disease of corn. *Phytopathology* **25**: 371–372.

Townsend, E. M, L. Kelly, L. Gannon, G. Muscatt, R. Dunstan, S. Michniewski, H. Sapkota, S. J. Kiljunen, A. Kolsi, M. Skurnik, T. Lithgow, A. D. Millard, E. Jameson. (2021). Isolation and characterization of *Klebsiella* phages for phage therapy. *Phage* **2**: 26-42. doi: 10.1089/phage.2020.0046.

Twort, F. W. (1936). Further investigations on the nature of ultra-microscopic viruses and their cultivation. *J. Hyg*. **36**: 204-35. doi: 10.1017/s0022172400043606.

Twort, F. W. (1915). An investigation on the nature of ultra microscopic viruses. *Lancet* **186**: 1241–1243. doi: 10.1016/S0140-6736(01)20383-3.

Umrao, P. D., V. Kumar, S. D. Kaistha (2021). Biocontrol potential of bacteriophage ϕsp1 against bacterial wilt-causing *Ralstonia solanacearum* in *Solanaceae* crops. *Egypt. J. Biol. Pest. Control*. **31**: 61. doi:10.1186/s41938-021-00408-3.

Verbeken, G., J. P. Pirnay (2022). European regulatory aspects of phage therapy: magistral phage preparations. *Curr. Opin. Virol*. **52**: 24-29. doi:10.1016/j.coviro.2021.11.005.

Vlassov, V. V., N. V. Tikunova, V. V. Morozova (2020). Bacteriophages as therapeutic preparations: what restricts their application in medicine. *Biochem. Mosc*. **85**: 1350–1361. doi: 10.1134/S0006297920110061.

Wang, J., X. Wang, K. Yang, C. Lu, B. Fields, Y. Xu, Q. Shen, Z. Wei, V. P. Friman (2023). Phage selection drives resistance-virulence trade-offs in *Ralstonia solanacearum* plant-pathogenic bacterium irrespective of the growth temperature. *Evol. Lett.* **8**: 253-266. doi:10.1093/evlett/qrad056.

Wang, J., S. Liang S, X. Lu, Q. Xu, Y. Zhu, S. Yu, W. Zhang, S. Liu, F. Xie (2023). Bacteriophage endolysin Ply113 as a potent antibacterial agent against polymicrobial biofilms formed by enterococci and *Staphylococcus aureus*. *Front. Microbiol*. **14**: 1304932. doi: 10.3389/fmicb.2023.1304932.

Wang, X., Z. Wei, K. Yang, J. Wang, A. Jousset, Y. Xu, Q. Shen, V. P. Friman (2019). Phage combination therapies for bacterial wilt disease in tomato. *Nat. Biotechnol*. **37**: 1513–1520. doi:10.1038/s41587-019-0328-3.

Wilkinson, L. (2001). Félix d'Herelle and the origins of molecular biology. *Med. Hist.* **45**: 294–295.

Wittmann, J., R. Eichenlaub, B. Dreiseikelmann (2010). The endolysins of bacteriophages CMP1 and CN77 are specific for the lysis of *Clavibacter michiganensis* strains. *Microbiology* **156**: 2366-2373. doi: 10.1099/mic.0.037291-0.

World Economic Forum Top 10 Emerging Technologies of 2023. Available online: https://www3.weforum.org/docs/WEF_Top_10_Emerging_Technologies_of_2023.pdf (accessed on April 02, 2024).

Wu, J., J. Liu, S. Liu, U. Handique, Q. Sun, Z. Feng, R. Zhang (2024). Isolation and characterization of lytic bacteriophages infecting *Pectobacterium atrosepticum*. *Eur. J. Plant Pathol*. **169**: 121-130. doi:10.1007/s10658-024-02814-3.

Yamamoto, N., D. Fraser, H. R. Mahler (1968). Chelating agent shock of bacteriophage T5. *J. Virol*. **2**: 944–950. doi:10.1128/JVI.2.9.944-950.1968.

Yan, J., R. Yang, S. Yu, W. Zhao (2021). The application of the lytic domain of endolysin from *Staphylococcus aureus* bacteriophage in milk. *J. Dairy Sci*. **104**: 2641-2653. doi:10.3168/jds.2020-19456.

Yang, Q., S. Le, T. Zhu, N. Wu (2023). Regulations of phage therapy across the world. *Front. Microbiol.* **14**: 1250848. doi: 10.3389/fmicb.2023.1250848.

Yang, Q., Y. Ding, R. Nie, L. Yao, X. Wang, M. Zhou, J. Wang, X. Wang (2020). Characterization of a novel T7-like *Salmonella Typhimurium* (ATCC13311) bacteriophage LPST144 and its endolysin. *L.W.T*. **123**: 109034. doi:10.1016/j.lwt.2020.109034.

Zaczek-Moczydłowska, M. A., G. K. Young, J. Trudgett, C. Plahe, C. C. Fleming, K. Campbell, R. O'Hanlon (2020). Phage cocktail containing *Podoviridae* and *Myoviridae* bacteriophages inhibits the growth of *Pectobacterium spp*. under in vitro and in vivo conditions. *PLoS One* **15**: 1-24. doi:10.1371/journal.pone.0230842.

Zbikowska, K., M. Michalczuk, B. Dolka (2020). The use of bacteriophages in the poultry industry. *Animals* **10**: 872. doi:10.3390/ani10050872.

Zhang, H., X. Zhang, S. Liang, J. Wang, Y. Zhu, W. Zhang, S. Liu, S. Schwarz, F. Xie (2023). Bactericidal synergism between phage endolysin Ply2660 and cathelicidin LL-37 against vancomycin-resistant *Enterococcus faecalis* biofilms. *NPJ Biofilms Microbiomes* **9**: 16. doi:10.1038/s41522-023-00385-5.

Zhang, X., Y. D. Niu, Y. Nan, K. Stanford, R. Holley, T. McAllister, C. Narváez-Bravo (2019). SalmoFresh™ effectiveness in controlling *Salmonella* on romaine lettuce, mung bean sprouts and seeds. *Int. J. Food Microbiol*. **305**: 108250. doi: 10.1016/j.ijfoodmicro.2019.108250