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Elucidating The Role Of Bacteriophages In Plant Disease Management And Market Scenario

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Abstract

Plants are essential for human diets and food security but face significant threats from bacterial diseases, which impact agricultural productivity. Managing these diseases is challenging due to limited bactericidal options, pathogen variability, and rapid bacterial mutation. Traditional treatments have involved antibiotics and copper-based compounds, which, despite being initially effective, have led to environmental issues and bacterial resistance. Phage therapy, using bacteriophages that specifically target bacterial pathogens, offers a promising, eco-friendly alternative. Bacteriophages, found abundantly in nature, can selectively destroy specific bacterial strains without harming beneficial organisms, highlighting their potential for sustainable plant disease control. Phages are safe for eukaryotic cells, reinforcing their value in plant health management. Although phage therapy originated in the early 20th century, its use diminished after the discovery of broad-spectrum antibiotics. However, the rise of resistant bacterial strains has renewed interest in this approach. Recent research has advanced understanding in phage classification, structure, and host interaction mechanisms. Modern developments include the use of naturally occurring phages, engineered variants, and phage-derived enzymes, all showing effectiveness in controlling crop bacterial pathogens through diverse treatment strategies. This review emphasizes the historical context, structural details, and applications of phage therapy, illustrating its potential to replace conventional bactericides and contribute to sustainable agricultural practices.

Keywords: Phage therapy, Bacterial diseases, Sustainable agriculture, Bacteriophages, Plant disease management, Antibiotic resistance.

Introduction:

Plants, crucial for human diets and food security, often face threats from various pests and diseases like fungi, viruses, and bacteria, leading to significant agricultural losses (Agrios, 2005). Managing bacterial diseases in crops poses particular challenges due to limited effective bactericides, pathogen variability, and high mutation rates (Balogh *et al*., 2010). Traditionally, chemical-based treatments like

antibiotics and copper have been used extensively but have led to the emergence of resistant strains and environmental concerns (McManus *et al*., 2002; Svircev *et al*., 2018). Consequently, eco-friendly methods have been developed, including the use of bacteriophages, viruses that target and destroy specific bacteria, known as phage therapy (Calvo-Garrido *et al*., 2014; Wiesel *et al*., 2014). Bacteriophages offer two major advantages: they have a narrow host range, targeting specific bacteria without affecting others, and they have no known negative effects on eukaryotic cells, making them potential tools for both plant disease management and human disease prevention (Farooq *et al*., 2018; Loc-Carrillo and Abedon, 2011). This dual utility highlights their promise in sustainable agriculture and public health efforts (Nagai *et al*., 2017).

Bacteriophages, or phages, are the most abundant viruses on Earth, infecting bacteria and archaea (Clokie *et al*., 2011). The term "bacteriophage" combines "bacterio," from "bacteria," and "phage," from the Greek "phagein," meaning "to devour" or “to nibble” or “to eat” (Sakib *et al*., 2021). These self-replicating, obligatory intracellular parasites are inert biochemically in the extracellular environment but control the biosynthetic machinery of bacterial hosts, prompting them to produce viral proteins. Phages are essentially particles containing nucleic acid (DNA or RNA), encoding the necessary information for replication. They exhibit a strong affinity for bacteria during infections and are widespread across various environments. Their abundance correlates directly with the bacterial population. It's believed that there are over 10^{30} tailed phages in existence (Brussow and Hendrix, 2002). Phages are prevalent in soil and easily obtained from feces and sewage. They are also abundant in freshwater and oceans, with an estimated 10 million virus-like particles per milliliter of seawater (Breitbart, 2012; Suttle, 2007). The capacity of bacteriophages to eliminate bacteria suggests a broad potential for their use as a substitute for antibiotics. The application of lytic phages or their derivatives in treating bacterial infections is referred to as phage therapy.

Early History and Researches on Bacteriophages

Hankin (1896) discovered a substance in the Ganges and Yamuna rivers that could pass through a fine porcelain filter and had antibacterial effects against cholera. The pioneer in phage therapy was Ferick Twort (1915) and Felix d'Hellex (1917), who observed some small agents parasitizing bacteria in growing culture and named as "bacteriophage". It was recognized as potential antimicrobial agents soon after that (Hermoso *et al*., 2007). However, the interest in phage therapy was rapidly reversed and displaced by the discovery into new broad-spectrum antibiotics in the 1940s. Bacteriophages, in terms of association with plant pathogenic bacteria, were first discovered by Mallmann and Hemstreet, who demonstrated the inhibited growth of *Xanthomonas campestris* pv. *campestris*, by treatment with filtered decomposed cabbage (Mallmann and Hemstreet, 1924). Subsequently, Kotila and Coons suggested that the isolated bacteriophages could prevent soft rot on slices of potato tuber and carrot caused by *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subsp. *carotovorum*, respectively (Kotila and Coons, 1925). Bacteriophage treatment of corn seeds was first shown to reduce the incidence of Stewart's wilt disease by 16.5% (Thomas, 1935). However, early field studies found bacteriophage treatment less effective than newly discovered broad-spectrum antibiotics (Goto, 2012), leading to waning interest in phage therapy despite promising results. Consequently, antibiotics and bactericidal chemicals have remained primary components of bacterial plant disease management strategies for decades (Agrios, 2005), despite concerns about their negative environmental and health impacts (Hermoso *et al*., 2007). Moreover, the prevalence of antibiotic, pesticide or copper-resistant bacteria such as *Erwinia amylovora* (Manulis *et al*., 2000), *Pseudomonas syringae* (Hwang *et al*., 2005; Masami *et al*., 2004), *Xanthomonas campestris* pv. *juglandis* (Lee *et al*., 1994), *Xanthomonas citri* spp. *citri* and *Xanthomonas alfalfa* spp. *citrumelonis* (Behlau *et al*., 2011), as well as very slow development of new effective antibiotics, twisted scientist attention toward other potential biocontrol agents for control of bacterial plant diseases. Stonier *et al*. (1967) reported that fewer than 10 bacteriophage particles showing the clear plaques present at the beginning of 21-h induction period were able, to inhibit completely tumor induction by highly virulent *Agrobacterium tumefaciens* corn strain B6.

Classification

After the advent of electron microscopy, Ernst Ruska proposed the first phage classification in 1943, distinguishing three morphological types (Ruska, 1943). Holmes classified phages within the order Virales in 1948, based on host range, but this system was not widely accepted (Holmes, 1948). In 1962, Lwoff, Horne, and Tournier proposed classifying viruses based on nucleic acid type (DNA or RNA), capsid shape, envelope presence, and capsomer count. Tailed phages were assigned the order rank Urovirales (Lwoff *et*

al., 1962). In 1966, a virus classification committee was established, shifting focus from host range and pathogenicity to virion and nucleic acid properties (P.C.N.V, 1965). This committee evolved into the ICTV (International Committee of Taxonomy of Viruses), which has published eighth reports in the year 2005 (Fauquet *et al*., 2005). For a deeper exploration of phage classification's development and challenges, further information can be found elsewhere (Ackermann, 2005). Phage classification traces back to Bradley's 1967 scheme, featuring six basic morphological types exemplified by phages T4, λ, T7, φX174, MS2, and fd (Bradley, 1997). Over time, new families and genera augmented the classification, resulting in one order, 14 families, and 37 genera (Fauquet *et al*., 2005). The ICTV embraces the polythetic species concept, defining a species by a varied set of properties (Van Regenmortel, 1990). With new phages continually being discovered, classification remains open-ended, with the ICTV lagging behind in its classification schedule. Over 5,500 phages with known morphology have been identified (Ackermann, 2007). ICTV has categorized 19 phage families, with the most extensively studied ones including Myoviridae, Siphoviridae, Podoviridae, Inoviridae, Microviridae, and more recently identified families like Ackermannviridae and Herelleviridae. These families are all classified within the order Caudovirales (Adriaenssens *et al*., 2018; Walker *et al*., 2019). Phages encompass viruses with various nucleic acid types: double-stranded DNA (dsDNA; the most common), single-stranded DNA (ssDNA), single-stranded RNA (ssRNA), and double-stranded RNA (dsRNA; rare). The majority of virions (96%) are tailed, while other types (referred to as CFP) include "cubic," filamentous, or pleomorphic forms (about 4% of representatives). "Cubic" indicates cubic symmetry and an icosahedral shape. Some phages have lipid envelopes or internal components, which are typically sensitive to ether and chloroform. CFP phage groups are mostly small and often have only a single representative.

Table 1: Overview of Prokaryote Viruses

Shape	Nucleic acid	Family	Genera	Particulars	Example	Members
Tailed	dsDNA (L)	Myoviridae	6	Tail contractile	T4	1320
		Siphoviridae	7	Tail long, noncontractile	λ	3229
		Podoviridae	4	Tail short	T7	771
Polyhedral	ssDNA (C)	Microviridae	4	Conspicuous capsomers	φX174	40
	dsDNA (C,S)	Corticoviridae	1	Complex capsid, lipids	PM2	3?
	dsDNA (L)	Tectiviridae	1	Double capsid, lipids, pseudo-tail	PRD1	19
	dsDNA (L)	SH1∗	-	Double capsid, lipids	SH1	1
	dsDNA (C)	STIV∗	-	Turret-shaped protrusions	STIV	1
	ssRNA (L)	Leviviridae	2	Poliovirus-like	MS2	39
	dsRNA (L, M)	Cystoviridae	1	Envelope, lipids	φ6	3
Filamentous	ssDNA (C)	Inoviridae	2	Long filaments, short rods	M13	67
	dsDNA (L)	Lipothrixviridae	4	Envelope, lipids	TTV1	7
	dsDNA (L)	Rudiviridae	1	Stiff rods, TMV-like	SIRV-1	3
Pleomorphic	dsDNA (C,S)	Plasmaviridae	1	Envelope, no capsid, lipids	L2	5
	dsDNA (C,S)	Fuselloviridae	1	Lemon-shaped, envelope, lipids?	SSV1	11
	dsDNA (L,S)	-	1**	Lemon-shaped, envelope	His1	1

	dsDNA (C,S)	Guttaviridae	1	Droplet-shaped	SNDV	1
	dsDNA (L)	Ampullaviridae∗		Bottle-shaped, helical NC	ABV	1
	dsDNA (C)	Bicaudaviridae∗		Two-tailed, development cycle, helical NC	ATV	1
	dsDNA (L	Globuloviridae∗		Envelope, spherical, lipids, helical NC	PSV	1

C, circular; L, linear; M, multipartite; NC, nucleocapsid; S, supercoiled; —, no name; ∗, non-classified; ∗∗, genus Salterprovirus. Members indicate numbers of phages examined by electron microscopy, excluding phage-like bacteriocins and known defective phages (based on computations from January 2006; Ackermann, 2007)

Structures

Bacteriophage structures are diverse, but most of them share some common characteristics. Bacteriophage T_4, a well-studied model organism, provides valuable insights into the characteristics and behavior of bacterial viruses. Its structure consists of a head and a tail-like appendage. The head is the site where the double-stranded DNA of the phage is housed, although the exact mechanism by which this DNA is packed inside the head remains a subject of study. The head itself takes on the shape of two halves of an icosahedron connected by a short, hexagonal prism. This geometric arrangement allows for efficient storage of genetic material within the confines of the phage's head. The tail of bacteriophage T_4 exhibits a helical structure. One notable feature of the protein coat of the tail is its binal symmetry, characterized by two distinct types of symmetry patterns. This binal symmetry is a defining trait of bacteriophages and cyanophages, distinguishing them from other types of viruses. The tail structure consists of a hollow tube that is cubical in shape and is surrounded by a contractile sheath. One end of the tail is attached to the head via a collar, while the other end features a hexagonal plate. This plate is equipped with six small fibers, known as 'tail pins', located at each corner, along with six tail fibers. These tail fibers serve crucial roles during the infection process. The long tail fibers are primarily responsible for the initial attachment of the phage to the bacterial cell wall. They recognize specific receptors on the surface of the host bacterium, facilitating the initial binding of the phage to its target. On the other hand, the short tail fibers play a critical role in anchoring the phage firmly to the host bacterium during the subsequent stages of infection. They help to stabilize the phage attachment site, particularly during the contraction of the sheath that occurs prior to DNA injection into the host cell. During the process of adsorption, the phage enzyme likely facilitates the dissolution of a pore in the bacterial cell wall, allowing for the injection of the phage DNA into the host cell. This marks the beginning of the infection process, which can lead to one of three distinct life cycles: the lytic cycle, exemplified by the T series of phages infecting Escherichia coli, the lysogenic cycle and Chronic cycle. These cycles determine the fate of the infected bacterial cell and the subsequent replication and spread of the phage genome.

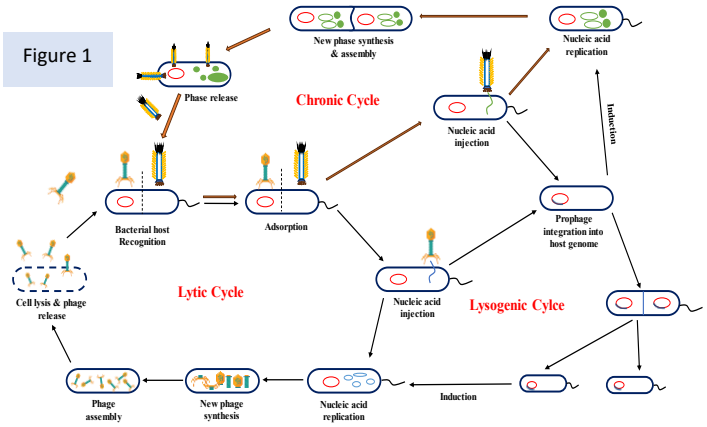
Bacteriophages-host interaction

Bacteriophages rely entirely on host cells for their reproduction, utilizing host cell components for replication. Bacteriophages typically exhibit three distinct life cycles: lytic, temperate (lysogenic), and a third cycle referred to as the "chronic cycle" (Howard-Varona *et al*., 2017), as illustrated in Figure 1. The lytic cycle of phages regulates the lysis of bacterial cells, hence they are also known as virulent phages (Vu and Oh, 2020). The infection process of bacteriophages initiates with the identification of bacterial cells, followed by the attachment of bacteriophages to specific receptors on the cell surface of susceptible bacteria, and subsequently, the transfer of phage nucleic acid into the host cell. Within the host cell, phages replicate and generate numerous new phage virion particles (Stone *et al*., 2019). During the lytic phase, phages produce the protein holin and the enzyme endolysin to induce lysis of bacterial cells and release the phage progeny (Dy *et al*., 2018). Generally, high concentrations of lytic phages are necessary against particular bacterial pathogens to enhance effectiveness and prevent the development of resistance in the bacterial host.

In the lysogenic cycle, the injected phage genomic DNA integrates into the bacterial chromosome or plasmid as a prophage. Prophages replicate along with the bacterial chromosome and are transmitted to daughter cells. Prophages can switch to a lytic cycle spontaneously, causing the death of their host,

particularly when bacterial cells are exposed to environmental stresses such as antibiotics and certain metabolic conditions of the host bacteria (Nanda *et al*., 2015; Davies *et al*., 2016).

Figure 1



A third mechanism, recently defined as the chronic cycle, involves phage-host interaction resulting from chronic infection, observed in filamentous phages belonging to the Inoviridae family (Yamada, 2013). In the chronic cycle, phages do not cause lysis of host cells but instead establish a persistent association with the host, continuously producing and releasing phage particles from growing and dividing host cells (Howard-Varona *et al*., 2017; Horiuk *et al*., 2020). In this scenario, phages establish a non-lethal chronic infection with ongoing phage production (Yamada, 2013; Sieiro *et al*., 2020). Infection of *Ralstonia solanacearum* with filamentous ϕRSS1 phage resulted in abnormal behavior, including reduced turbidity and frequent aggregation in liquid culture; intriguingly, ϕRSS1-infected cells exhibited enhanced virulence on tobacco (Yamada *et al*., 2007) and tomato plants (Addy *et al*., 2012; Yamada, 2013). However, another filamentous phage, ϕRSS0, decreased the virulence of R. solanacearum (Yamada, 2013).

A variation of the lysogenic cycle is known as the carrier state or pseudolysogenic cycle, wherein the phage's nucleic acid does not replicate but remains dormant within the host. Pseudolysogeny likely occurs during periods of cellular starvation when there is insufficient energy available for viral gene expression. Upon the reintroduction of nutrients, the pseudolysogenic state is resolved, either by initiating the lytic cycle or by establishing true lysogeny (Cenens *et al*., 2013).

Receptors used by phage to detect bacteria

Many diverse molecular structures on the surface of bacteria can act as phage receptors, but their nature and position on the cell differs with specific bacteria–phage interactions. The receptors can be protein, polysaccharide, lipopolysaccharides (LPS) and carbohydrate moieties (Bertozzi Silva *et al*., 2016). In Gram-negative bacteria, LPS is a common receptor for phages. In addition, other receptors are outer membrane proteins, pili and flagella (Sorensen *et al*., 2011). The exact mechanisms of action have been well studied in the model E. coli myovirus T4. This phage attaches reversibly to LPS or to the outer membrane protein porin OmpC, depending on the strain of E. coli. This attachment then leads to irreversible binding of T4 with the outer core region of *E. coli* LPS. The model E. coli phage T7 also uses LPS as the main irreversible phage binding site (Sorensen *et al*., 2011). For phages that infect Gram-positive bacteria, peptidoglycan is an important phage receptor, as it is a major polymer on bacterial surfaces, along with teichoic acids, that are attached covalently to the peptidoglycan layer. Polysaccharides that are exposed on the surface of the bacteria are also common receptors (Bertozzi Silva *et al*., 2016). In many ways, it is surprising that only a small 7 number of phage receptors have been identified for Gram-positive bacteria; this is, in part, due to their complex outer structure and, in part, due to the scarcity of research activities on phages that target Gram-positive bacteria in general. An example of a Gram-positive bacteria phage with a known receptor is the phage 3C that attaches to the N-acetyl glucosamine moiety of teichoic acids on the surface of *Staphylococcus aureus*. Another example is the D-glucose chain of the teichoic acid on the *B. subtilis* surface, which functions as the receptor for phages SP2 and SP10 (Rakhuba *et al*., 2010).

Modern approaches to phage therapy

A spectrum of strategies have been described here ranging across natural and engineered phages, enzymes derived from phages and combination of phages with antimicrobial substances.

1. **Conventional Phage Therapy:** Conventional phage therapy involves the direct application of naturally isolated virulent phages to patients to target and lyse pathogenic bacteria. In a study by Bruttin and Brussow, 15 adult volunteers received E. coli T4 phage orally, with mild adverse reactions reported by only a few volunteers, none requiring treatment. This approach demonstrates promising evidence and a favorable safety profile.

2. **Modified Phage:** Genetic engineering is employed to create modified phages with broader host ranges and lacking toxin genes. These bioengineered phages can deliver lethal genes or substances to target bacteria without lysing host cells. For instance, non-lytic filamentous phages have been successfully modified to deliver genes encoding lethal proteins, such as restriction endonucleases or addiction toxins, which induce apoptosis in target bacteria.
3. **Phage-Derived Proteins:** Phage-encoded enzymes offer an alternative to whole phage therapy. These enzymes play crucial roles in rupturing bacterial cells during infection. Virion-Associated Peptidoglycan Hydrolases (VAPGH) and endolysins are two groups of phage proteins involved in this process. Endolysins, in particular, have shown efficacy against various Staphylococcal infections, including MRSA, MSSA, VRSA, and VISA, in vitro. They act as specific bacteriolytic agents at low dosages, offering a promising avenue for targeted treatment.
4. **Dual Therapy:** Phage Combined with Antibiotics Dual therapy, combining phages with antibiotics, is a promising strategy against antimicrobial resistant pathogens. This approach exploits synergistic effects, resulting in higher therapy success rates and reduced resistance emergence. Studies demonstrate in vitro inhibition of P. aeruginosa growth with combined phage and antibiotic (streptomycin) treatment.

Recent Uses of Bacteriophages in Managing Bacterial Diseases in Plants

1. **Individual Bacteriophages application:** Currently, most research on bacteriophages targeting bacterial plant pathogens has focused on their isolation and characterization, though some isolated phages show promise for therapeutic use (Rahimi-Midani *et al*., 2018; Yin *et al*., 2019). A survey in Molecular Plant Pathology identified the top ten most significant bacterial plant pathogens, with *Pseudomonas syringae* pathovars being particularly notable for their global impact (Mansfield *et al*., 2012). Field trials in 2016 tested a cocktail of six phages against bacterial blight in leeks caused by *P. syringae* pv. *porri*, showing mixed results but suggesting potential for phage therapy (Rombouts *et al*., 2016). Recent studies have also explored various application methods, including soil drenching, foliar spraying, and seed immersion. For instance, soil drenching effectively reduced wilting in tomatoes caused by *R. solanacearum* (Elhalag *et al*., 2018), while foliar spraying decreased disease incidence from *X. campestris* pv. *campestris*, *Xanthomonas euvesicatoria*, and *P. carotovorum* subsp. *carotovorum* (Nagai *et al*., 2017; Gašić *et al*., 2018). Additionally, filamentous phages like ΦRSM3 have shown potential by enhancing the expression of defense-related genes in tomatoes and reducing the virulence of *R. solanacearum* (Addy *et al*., 2012), while phage XacF1 caused notable reductions in various traits of *X. axonopodis* pv. *citri* (Ahmad *et al*., 2014).
2. **Bacteriophage application as mixtures or cocktails:** While some bacteriophages target multiple bacterial genera (Ahern *et al*., 2014), most are specific to certain strains within a single species due to the precise interaction between phage attachment structures and bacterial cell surface receptors (Sulakvelidze *et al*., 2001). Bacteria can quickly develop resistance to phages through mechanisms such as mutation, adsorption-blocking, and restriction-modification or CRISPR-Cas systems (Chopin *et al*., 2005; Ranjani *et al*., 2018). For example, *R. solanacearum* developed resistance approximately 30 hours after phage application (Fujiwara *et al*., 2011), and similar resistance was observed with Xoo-Sp2 phages after 16 to 17 hours (Dong *et al*., 2018). To address these limitations, using a mixture of lytic phages, known as a phage cocktail, can enhance host range and reduce the likelihood of resistance (Schmerer *et al*., 2014; Tewfike and Desoky, 2015). Phage cocktails have been successfully used against various bacterial plant pathogens, including *R. solanacearum* (Ramírez *et al*., 2020; Wang *et al*., 2019), *Xanthomonas species* (Ibrahim *et al*., 2017; Tewfike and Desoky, 2015), and *P. carotovorum* (Zaczek-Moczydłowska *et al*., 2020). To further combat bacterial resistance, a patented method involving host-range (h-) mutant phages has been developed. These h-mutant phages have a broader range of activity, including strains resistant to the original phages, while still targeting the wild-type bacteria (Le Roy, 1989). For example, a mix of five h-mutant phages effectively controlled bacterial blight in geraniums caused by *X. campestris* pv. *pelargonii* (Flaherty *et al*., 2001). This approach also reduced bacterial spot severity in tomatoes and improved yields compared to untreated or chemically treated plants (Flaherty *et al*., 2000).
3. **Bacteriophages application with Other Antimicrobial Agents**: Combining bacteriophages with other antimicrobial agents, such as plant systemic acquired resistance (SAR) inducers and antibiotics, has shown promise in reducing disease severity. In 2005, Obradovic *et al*., 2015 investigated the effects of combining SAR inducers with biocontrol agents on bacterial spot disease in tomatoes caused by *X. campestris* pv. *vesicatoria*. They found that using bacteriophages along

with the SAR inducer acibenzolar-S-methyl (ASM) not only mitigated the hypersensitive response induced by ASM but also provided excellent disease control. Similarly, in field trials targeting bacterial leaf blight of onions caused by *X. axonopodis* pv. *allii*, a combination of a phage mixture and ASM reduced disease severity by 50%, outperforming a 31% reduction achieved with copper hydroxide-mancozeb treatment (Lang *et al*., 2007). Under greenhouse conditions, integrating phage KΦ1 with copper hydroxide significantly decreased the number of lesions on pepper leaves infected by *X. euvesicatoria* (reductions of approximately 81%, 90%, and 88% across three trials). Although copper hydroxide alone did not show a statistically significant difference in control efficacy, its combination with bacteriophages led to a notable reduction in lesion numbers (Gašić *et al*., 2018).

4. **Application of Bacteriophage-Derived Proteins—Endolysins:** Bacteriophage-derived endolysins, which are enzymes used to release new phage particles by degrading bacterial cell walls, offer several benefits over whole phages. These include a broader host range and reduced risk of resistance development. Endolysins are categorized into five types based on their enzymatic activities, such as N-acetylmuramidases and endo-β-N-acetylglucosaminidases, and can target either Gram-positive or Gram-negative bacteria. For example, endolysins from phages CMP1 and CN77 effectively lysed specific strains of *C. michiganensis* (Wittmann *et al*., 2010), while endolysins from phages Atu_ph02 and Atu_ph03 led to rapid lysis in *A. tumefaciens* (Attai *et al*., 2017). Endolysins also demonstrated broad activity against multiple bacterial species and even fungi when introduced into plants, enhancing resistance to pathogens like *Rhizoctonia solani* (Dong *et al*., 2008). Despite their effectiveness, endolysins face challenges with Gram-negative bacteria due to their protective outer membrane. To address this, strategies such as combining multiple endolysins or using depolymerases to disrupt bacterial barriers have been developed, improving the efficacy of phages against these pathogens (Born *et al*., 2014; Born *et al*., 2017).

Application of bacteriophages carried out during research

S. No.	Pathogen	Host	Disease	Information	References
01	*Pectobacterium carotovorum* ssp. *carotovorum*, *Pectobacterium wasabiae*, *Dickeya solani*	Potato	Soft Rot	Bioassays with phage 8PD10.3 and 8PD23.1 could reduce severity of soft rot of tubers by 80% on potato slices and 95% with whole tubers from a mixed pathogen infection.	Czajkowski *et al*., 2015
02	*Dickeya solani*	Potato	Soft rot/Black leg	Phage vB_DsoM_LIMEstone1 and vB_DsoM_LIMEstone2 reduced soft rot of inoculated tubers in bioassays and in field trials which produced a potato crop with higher yields.	Adriaenssens *et al*., 2012
		Potato	Soft Rot	Bioassays with phage 8D1, 8D2, 8D3, 8D4, 8D5, 8D7, 8D9, 8D10, 8D11 could reduce incidence of soft rot by up to 30–70% on co inoculated potato slices with pathogen and phage.	Czajkowski *et al*., 2014
03	*Ralstonia solanacearum*	Tomato	Bacterial spot	Tomato plants treated with phage 8RSL1 showed no symptoms of bacterial wilt during the experimental period; whereas all untreated plants showed wilting 18 days post infection.	Fujiwara *et al*., 2011
				Simultaneous treatment of phage PE204 with R. solanacearum of the rhizosphere of tomato completely inhibited bacterial wilt. However, pre-treatment with phage before the inoculation of pathogen was not effective with control of bacterial wilt, whereas post treatment of PE204 delayed disease development.	Bae *et al*., 2012
04	*Xanthomonas campestris* pv. *vesicatoria*	Tomato	Bacterial spot	Greenhouse experiments with formulated phage cocktails could reduce disease severity with formulated phage cocktails providing better protection in comparison to unformulated. A similar effect was found in three consecutive field trials.	Balogh *et al*., 2003
				In field experiments phage treatment was comparable to disease control with copper-mancozeb. Combination of phage and plant activator (ASM) resulted in enhanced control.	Obradovic *et al*., 2004
05	*Xylella fastidiosa*	Grapevine	Pierce's Disease	X. fastidiosa levels in grapevines were significantly reduced on pre and post inoculation of a four phage (Sano, Salvo, Prado and Paz)	Das *et al*., 2015

				cocktail. Pierce disease symptoms could be stopped using phage treatment post infection as well as applying phage prophylactically to grapevines.	
06	*Xanthomonas axonopodis* pv. *allii*	Onion	*Xanthomonas* leaf blight of onion	Field trial showed that weekly and biweekly applications of phage could reduce disease severity, a result which was comparable to treatments of weekly applications of copper mancozeb.	Lang *et al*., 2007
07	*Pectobacterium carotovorum* ssp. *carotovorum*	Lettuce	Soft Rot	Green house trials showed that phage PP1 could significantly reduce disease development on lettuce plants.	Lim *et al*., 2013
08	*Streptomyces scabies*	Radish	Common scab	Phages Stsc1 and Stsc3 could prevent disease development by treating radish seedlings. Non-treated radishes had 30% less weight than negative control, with phage treated radishes having masses similar to negative control.	Goyer, 2005
09	*Pseudomonas tolaasi*	Mushrooms	Brown blotch disease	Surface of mushrooms were inoculated with pathogen. The formation of blotches was completely blocked by co-incubation of phages with pathogen.	Kim *et al*., 2011
10	*Xanthomonas axonopodis* pv. *citri*	Grapefruit	Asiatic citrus canker	Five greenhouse experiments utilizing phage treatment could reduce disease severity by 59%. However, using a skim milk formulation of phage did not have increased disease control. Phage treatment was also capable of reducing disease occurrence in a citrus nursery. Control was less effective than copper-mancozeb. Combination did not give increased disease control.	Balogh *et al*., 2008
11	*Xanthomonas axonopodis* pv. *citrumelo*	Orange	Citrus bacterial spot	Phage treatments reduced citrus spot occurrence by 35 and 48% in two trials in commercial citrus nursery. Control was equal or less effective than copper mancozeb. Combination did not give increased disease control.	Balogh *et al*., 2008
12	*Pseudomonas syringae* pv. porri	Leek	Bacterial blight	Specific bio-assays demonstrated the in-planta efficacy of phages vB_PsyM_KIL1, vB_PsyM_KIL2, vB_PsyM_KIL3, and vB_PsyM_KIL3b. However, phage cocktail of six phages (vB_PsyM_KIL1, vB_PsyM_KIL2, vB_PsyM_KIL3, vB_PsyM_KIL4, and vB_PsyM_KIL5 and vB_PsyM_KIL3b),	Rombouts *et al*., 2016

				were tested with two parallel field trial experiments in three locations which showed variable results. In one trial, symptom development was attenuated.	
13	*Erwinia amylovora*	Pear, Apple	Fire blight	Phages 8Ea1337-26 and 8Ea 2345 reduced infection of detached pear tree blossoms by 84 and 96%, respectively, with Pantoea agglomerans as a carrier. Also, infection of potted apple tree blossoms could be reduced by 54% with phage 8Ea1337-26 and P. agglomerans. Control was comparable to streptomycin.	Boulé *et al*., 2011
14	*Acidovorax citrulli*	Melon	Bacterial fruit blotch	Phage application after symptom development resulted in 27% disease severity, compared to 80% for the non-treated control. Phage detected in foliar tissue 8 h after addition to soil and leaf tip after 24 h.	Rahimi-Midani and Choi, 2020
15	*Pectobacterium atrosepticum*	Potato	Soft rot	Use of the phage cocktail reduced both disease incidence and disease severity by 61% and 64% respectively.	Carstens *et al*., 2019
16	*Pectobacterium spp*. and *Pantoea spp*.	Onion	Soft rot	Over four years, both immersion and spray methods consistently reduced disease severity with uniform results.	Zaczek Moczydłowska *et al*., 2020
17	*Pseudomonas syringae* pv. *actinidiae*	Kiwifruit	Bacterial blight	Within 24 hours post-infection, phages reduced bacterial load on kiwifruit leaves by over 75%. No significant difference was noted between one or two applications, but phages outperformed copper bactericides in disease control.	Flores *et al*., 2020
18	*Ralstonia solanacearum*	Tomato	Bacterial wilt	A phage cocktail killed 98% of live bacteria in sterilized soil one week after spraying. Treatment effectiveness depended on timely application, with early use after initial bacterial wilt signs being crucial.	Wei *et al*., 2017
				Phages reduced disease incidence by up to 80% in field experiments. Increasing the number of phages in the cocktail further decreased disease severity.	Wang *et al*., 2019
19	*Ralstonia solanacearum*	Banana	Moko wilt	The phage cocktail provided complete (100%) protection against Moko disease, whereas plants treated with single-phage treatments showed disease symptoms.	Ramírez *et al*., 2020

20	*Xanthomonas oryzae* pv. *oryzae*	Rice	Bacterial blight	Spraying rice seedlings with phages 2, 4, 6 days post-inoculation reduced disease severity by 73.9%, 49.6%, and 28.9%, respectively. Pre-inoculation phage spraying reduced severity by 83.1%, while seed treatment achieved a 95.4% reduction.	Ogunyemi *et al*., 2019
				The phage formulation with skim milk reduced bacterial leaf blight occurrence to 18.1%, compared to 87% in the untreated control.	Chae *et al*., 2014
21	*Xanthomonas euvesicatoria*	Pepper	Bacterial Spot	The most effective disease control was achieved with two phage applications (before and after inoculation). However, the best results came from integrating phage application 2 hours before inoculation with copper hydroxide applied 24 hours prior.	Gašić *et al*., 2018
22	*Xanthomonas citri* subsp. *citri*	Citrus	Canker	Non-formulated phages with ASM reduced disease incidence by 42.4–56.9%, while formulated phages combined with ASM achieved a reduction of 82.1–86.1%.	Ibrahim *et al*., 2018
23	*Xanthomonas campestris* pv. *campestris*	Broccoli	Black rot	Only the nonpathogenic *Xanthomonas sp*. strain mixed with bacteriophage reduced disease; phage alone had no effect.	Nagai *et al*., 2017
24	*Ralstonia. solanacearum*	Tomato	Bacterial wilt	Adding phage suspension one day before bacterial inoculation effectively controlled the disease.	Elhalag *et al*., 2018

List of phage products available for bacterial disease control

S.No.	Product Name	Country	Active Ingredient (Bacteriophage Against Pathogen)	PFU/ml	Use for Diseases and Crop	Company	References
01	Agriphage	USA	*Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. tomato phage	1.55 × 1013	Bacterial leaf spot of tomato and pepper	OmniLytics, Inc	OmniLytics, n.d. b
02	AgriPhage-CMM	USA and Canada	*Clavibacter michiganensis* subsp. *michiganensis* phage	3.8 × 1012	Canker of tomato	OmniLytics, Inc	OmniLytics, n.d. a
03	Agriphage-Fire blight	USA	*Erwinia amylovora* phage	5 × 1012	Fire blight of apple and pear	OmniLytics, Inc	OmniLytics, 2018
04	Agriphage-Citrus canker	USA	*Xanthomonas citri* subsp. citri phage	5 × 1012	Canker of citrus	OmniLytics, Inc	OmniLytics, n.d. c
05	BioLyse-PB	United Kingdom	Soft rot bacteria of potato (Enterobacteriacea) phage		Soft rot of potato	APS Biocontrol	APS Biocontrol,n.d.
06	Erwiphage Plus	Hungary	*Erwinia amylovora* phage	2 × 105	Fire blight of apple, pear, quinces and loquat	Enviroinvest	Enviroinvest, n.d.
07	Xylphi-PD	USA	*Xylella fastidiosa* phage	5 × 109	Pierce's disease of grape	A&P Inphatec, n.d	A&P Inphatec, n.d

Potential Challenges of Bacteriophages for Bacterial Disease Management in Plants

Despite promising results in laboratory assays, field application of bacteriophages for plant disease management faces significant challenges. Bacteriophages are typically applied to the rhizosphere or sprayed on the phyllosphere. However, their success is influenced by several factors:

1. **Rhizosphere Challenges**: Limited water availability is essential for phage diffusion in soil. Bacteriophages can become trapped in biofilms or adsorbed to soil particles, reducing mobility. Low soil pH may inactivate phages, hindering their ability to find suitable hosts (Gill & Abedon, 2003).
2. **Phyllosphere Challenges**: The phyllosphere environment exposes bacteriophages to high temperatures, pH extremes, and UV radiation, which rapidly reduce their populations (Galić *et al*., 2018). Field studies have shown significant reductions in phage populations within 36-48 hours post-application (Dewlike & Deseky, 2015).
3. **Stability and Formulation**: Formulation strategies, such as encapsulating bacteriophages in materials like corn flour, skim milk, and lignin, have improved their stability (Arthurs *et al*., 2006). Applying treatments during early morning or evening has also shown promise (Iriarte *et al*., 2007).
4. **Field Efficacy vs. Laboratory Results**: In vitro characteristics, such as host range and lytic activity, do not always correlate with field efficacy (Bar *et al*., 2012; Bhuta, 2015). Some studies demonstrated that bacteriophages with strong in vitro activity did not achieve significant disease control in field trials (Maniats *et al*., 2016).
5. **Case Studies**: The use of RSL1, a bacteriophage with slower in vitro lytic activity, effectively controlled R. solanacearum in tomatoes and provided protection for up to 4 months, unlike more lytic counterparts (Tanaka *et al*., 1990). Additionally, filamentous phage XacF1, which forms small plaques, impacted host virulence by reducing extracellular polysaccharide production and mobility (Abmel *et al*., 2014).

Future Perspectives of Bacteriophage Usage in Plants

1. **Field Trial Needs**: Most successful bacteriophage applications are in controlled environments; more field trials are essential for open-field validation (Buttimer *et al*., 2017).
2. **Limited Commercial Products**: Few bacteriophage-based products, such as AgriPhages and Erwiphage, exist despite promising studies (Buttimer *et al*., 2017).
3. **Environmental Impact**: Field efficacy is reduced by environmental variability, emphasizing the need for robust delivery methods and formulations (Buttimer *et al*., 2017).
4. **Selection Criteria**: Standardized criteria for selecting effective phages need improvement, as current methods can have limitations (Ahmad *et al*., 2014; Rombouts *et al*., 2016).
5. **Potential of Temperate Phages**: While temperate phages are less commonly used due to replication risks, they could be engineered for beneficial uses, such as disrupting virulence (Halugh *et al*., 2010).
6. **Phage Detection**: Engineered phages can introduce marker genes for detecting pathogens, effective for both lytic and lysogenic types (Paroon *et al*., 2018).
7. **Transgenic Plants**: Phage protein expression has shown enhanced plant resistance, but regulatory and consumer concerns need addressing (Dong *et al*., 2008; Wittmann *et al*., 2016).
8. **Sustainability Potential**: Bacteriophages could reduce reliance on agrochemicals, supporting sustainable agriculture (Buttimer *et al*., 2017).

Conclusion

The resurgence of interest in phage therapy represents an important shift toward sustainable and targeted approaches for managing bacterial plant diseases. The historical success, recent advances, and application strategies underscore the potential of bacteriophages as viable alternatives to conventional treatments. This method's environmental compatibility and specificity provide significant advantages, especially in an era marked by increasing bacterial resistance to antibiotics and chemical treatments. Although phage therapy presents challenges, including bacterial resistance development, its combination with antibiotics and genetic engineering shows promising synergy and enhanced efficacy. Further research, field trials, and regulatory frameworks will be essential to integrate phage therapy into mainstream agricultural practices effectively.

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