J.Mycopathol.Res. 64(3) : 517-527, 2026, ISSN : 0971-3719 (Print), 2583-6315 (Online) doi.10.57023/JMycR.64.3.2026.517

REVIEW

Role of Bacteriophages in managing bacterial phytopathogens

NIKITA NEGI[1], VIJAY KUMAR[1], SATAKSHI NAUTIYAL[2], AAUSHI PANT[3], ASHWANI RAWAT[4], SHIWALI DHIMAN[5]

[1]Department of Plant Pathology, College of Horticulture, VCSG UUHF, Bharsar, Pauri Garhwal 246123, Uttarakhand
[2]Department of Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, NauniSolan 173230 Himachal Pradesh
[3]Department of Plant Pathology, School of Agricultural Sciences, Shri Guru Ram Rai University, Dehradun, 248001, Uttarakhand
[4]Department of Agriculture, School of Agriculture, Dev Bhoomi Uttarakhand University, 248007, Uttarakhand
[5]KrishiVigyan Kendra Sirmour (Dhaulakuan), CSKHPKV, Palampur 173031 Himachal Pradesh

Received : 08.05.2026 Accepted : 12 .08.2026 Published : 28.09.2026

Bacterial plant diseases are a major constraint to crops, fruits and vegetable production worldwide, leading to yield losses, reduced quality and economic loss. Conventional management strategies based on copper compounds and antibiotics have become increasingly limited due to the emergence of resistant bacterial populations, environmental contamination, and growing concerns regarding antimicrobial resistance. Bacteriophages, viruses that specifically infect and lyse bacteria, have emerged as environmentally safe and highly targeted biological control agents with considerable potential for sustainable disease management. Their high host specificity, self-replicating nature, and compatibility with integrated disease management make them attractive alternatives to chemical bactericides. Lytic bacteriophages are preferred over lysogenic phages because they rapidly eliminate bacterial pathogens without transferring undesirable genetic material. Compared with single-phage applications, phage cocktails consisting of multiple compatible lytic phages provide broader host coverage, improve disease suppression, and reduce the likelihood of resistance development. Successful application strategies include foliar sprays, soil drenching, seed treatments, and trunk injections, while advances in formulation technologies and phage engineering are enhancing stability and field performance. Several commercial bacteriophage products have already demonstrated effectiveness against economically important bacterial diseases in crops. However, factors such as environmental instability, narrow host range, formulation challenges, regulatory limitations, and large-scale production continue to influence their widespread adoption. Continued improvements in phage selection, cocktail design, formulation technologies, and integration with other sustainable disease management practices are expected to enhance the reliability and practical application of bacteriophage-based biocontrol, supporting environmentally friendly and long-term management of bacterial plant diseases

Keywords : Phytopathogen, antibiotics, bacteriophage, integrated disease management, virulence, biocontrol

INTRODUCTION

Bacterial phytopathogens continue to pose a major threat to global crop production because of their high virulence, adaptability and the limited effectiveness of conventional control measures.

Although bacteriophages have emerged as promising biological control agents most published studies have focused on individual phages, specific pathosystems or experimental evaluations.Information on the development, formulation, optimization and field application of phage cocktails remains scattered across the literature. In addition, recent advances in engineered phages, formulation technologies, resistance management strategies, and

*Correspondence : vijaykumar.india28@yahoo.in

commercially available bacteriophage products have not been comprehensively synthesized in a single review. This lack of an integrated and up-to-date assessment makes it difficult to identify current challenges, research priorities, and opportunities for practical implementation.

Therefore, this review aims to provide a comprehensive overview of bacteriophage-based biocontrol, with particular emphasis on the role of lytic phage cocktails in the management of bacterial plant diseases. The review discusses the biology of bacteriophages, mechanisms of bacterial control, formulation strategies, commercial applications, current limitations, and recent technological advances. By integrating recent findings and identifying existing knowledge gaps, this review highlights future research directions and the potential of phage cocktails as sustainable components of integrated plant disease management.

Among bacterial phytopathogens, several species are recognized as the most destructive because of their wide host range, economic impact, adaptability, and the challenges associated with their management. A landmark survey published in *Molecular Plant Pathology* ranked the ten most important bacterial plant pathogens based on scientific and economic significance. These include *Pseudomonas syringae* (tomato speck), *Ralstonia solanacearum* (bacterial wilt), *Agrobacterium tumefaciens* (crown gall), *Xanthomonas oryzae* pv. *oryzae* (bacterial leaf blight of rice), *Xanthomonas campestris* (black rot and wilt), *Xanthomonas-saxonopodis*pv. *manihotis* (cassava bacterial blight), *Erwiniaamylovora* (fire blight of apple and pear), *Xylella fastidiosa* (Pierce's disease of grapevine), *Dickeyadadantii* and *D. solani* (wilt and soft rot), and *Pecto bacterium carotovorum* and *P. atrosepticum* (soft rot). These pathogens were selected because of their global economic importance, high virulence, difficulty in management, and increasing resistance to conventional control measures. Since the publication of this ranking, advances in genomics, pathogen biology, and sustainable disease management have further emphasized the need for alternative approaches, including bacteriophage-based biocontrol, to manage these economically important bacterial diseases (Mansfield *et al*. 2012; Ryan *et al*. 2021; Farooq *et al*. 2022).

Present Approaches in Management

Copper compounds and a limited number of antibiotics are commonly used for the management of bacterial phytopathogens. Antibiotics such as tetracycline and kasugamycin have been used in some regions to control bacterial diseases in crops. However, their repeated use has contributed to the emergence of antibiotic-resistant bacterial populations, reducing their long-term effectiveness (McManus *et al*. 2002; Sundin and Wang, 2018). Similarly, the extensive application of copper-based compounds can adversely affect the environment by causing phytotoxicity, promoting the bioaccumulation of copper in soils, and reducing microbial diversity (Lamichhane *et al*. 2018; Farooq *et al*. 2022). Owing to concerns regarding antimicrobial resistance, environmental persistence, and ecological toxicity, the use of several antibiotics and certain pesticide formulations has been restricted or banned in many countries, driving the search for safer and more sustainable alternatives for the management of bacterial plant diseases (Sundin and Wang, 2018; FAO & WHO, 2019; Halawa *et al*. 2023).Considering the detrimental effects of pesticides on crop productivity, environmental health, and non-target organisms, there is an urgent need to develop alternative plant disease management strategies with improved efficacy and sustainability (Elnahal*et al*. 2022; Farooq *et al*. 2022). One of the most promising approaches is the use of biological control agents (BCAs), which provide effective disease management with minimal adverse environmental impact. Biological control involves the use of living organisms or their products to suppress plant pathogens and reduce disease incidence. Biocontrol agents, including bacteria, fungi, bacteriophages, and other beneficial microorganisms, inhibit the growth, survival, and reproduction of phytopathogenic bacteria through diverse mechanisms. These mechanisms may involve direct antagonism, such as parasitism, predation, or antimicrobial metabolite production, as well as indirect mechanisms, including competition for

nutrients and ecological niches, induction of plant defense responses, and manipulation of the microbial community. Owing to their eco-friendly nature, target specificity, and compatibility with integrated disease management strategies, biological control agents have emerged as sustainable alternatives to conventional chemical pesticides (Halawa *et al*. 2023).

Bacteriophages: What? Where? How?
What are Bacteriophages?

The use of bacteriophages as biological control agents (BCAs) has gained considerable attention for the sustainable management of bacterial phytopathogens (Pandit *et al*. 2022; Farooq *et al*. 2022). The term *bacteriophage* is derived from the Greek words *bakterion* (bacteria) and *phagein*(to devour), meaning "bacteria eater." Bacteriophages are viruses that specifically infect bacteria without causing harm to plants, animals, or humans. They also infect members of the domain Archaea.They are obligate intracellular parasites and acellular infectious entities. They typically range from 20 to 200 nm in size and are approximately 50 times smaller than bacterial cells. The largest known bacteriophage, *Bacillus megaterium* phage G (a jumbo phage), possesses a capsid approximately 160 nm in diameter, a tail about 453 nm long, and a genome of nearly 497 kb. Owing to their small size, phages are routinely visualized using transmission electron microscopy. They exhibit remarkable diversity in morphology, genome organization, and host range. As non-motile particles, bacteriophages rely on Brownian motion and fluid movement to encounter susceptible bacterial hosts. Most phages display high host specificity, infecting only a particular bacterial species or even specific strains within a species, making them attractive candidates for targeted biological control.Historically, most tailed double-stranded DNA bacteriophages were classified under the order *Caudovirales* and grouped into the families *Myoviridae*, *Siphoviridae*, and *Podoviridae* based on tail morphology. However, recent genome-based taxonomic revisions by the International Committee on Taxonomy of Viruses (ICTV) have replaced this morphology-based classification with several new families. Nevertheless, the traditional classification remains widely used for describing phage morphology, where *Myoviridae* possess contractile tails, *Siphoviridae* have long flexible non-contractile tails, and *Podoviridae* are characterized by short tails (Turner *et al*. 2023; ICTV, 2024).The existence of bacteriophages was first suggested by Sir Ernest Hankin in 1896, who observed that the water of the Ganges River possessed antibacterial activity against *Vibrio cholerae*. Later, bacteriophages were independently discovered by Frederick W. Twort in 1915 and Félix d'Herelle in 1917, with d'Herelle introducing the term "bacteriophage." In 1919, d'Herelle demonstrated their therapeutic potential by successfully treating patients suffering from bacterial dysentery (Leitner *et al*. 2021). The first report of bacteriophages in plant disease management was published in 1924 by Mallmann and Hemstreet, who demonstrated that filtrates from decomposed cabbage tissues inhibited the growth of *Xanthomonas campestris* pv. *campestris* (Nakayinga *et al*. 2021). Subsequently, the first successful field application of bacteriophages was reported by Thomas in 1935 for the management of Stewart's wilt of maize caused by *Pantoea stewartii*, reducing disease incidence from 18% in untreated plants to 1.4% in phage-treated plants (Jones *et al*.2021).

Structure of Bacteriophage

Bacteriophages exhibit binal symmetry, consisting of an icosahedral head and a helical tail. Although phage morphology varies among different groups, the majority of bacteriophages used for plant disease management are tailed phages with this characteristic structure. A typical bacteriophage is composed of a polyhedral head (capsid), a short neck or collar, and a tail terminating in a baseplate, tail fibers, and tail spikes (Turner *et al*. 2023; Dion *et al*. 2020).The polyhedral head (capsid) is an icosahedral protein shell composed of capsomeres that encloses and protects the viral genome, which is usually double-stranded DNA. The capsid provides structural stability and safeguards the genetic material until it is delivered into the bacterial host.The neck or collar connects the head to the tail and provides structural stability. It plays an important role in phage assembly and facilitates the transfer of viral DNA from the capsid into the bacterial cell during infection.The tail is a hollow, helical structure that serves as a conduit

for genome delivery. In phages possessing contractile tails, such as members of the former *Myoviridae* group, the tail consists of an inner hollow tube surrounded by a contractile sheath. During infection, contraction of the sheath drives the inner tube through the bacterial cell envelope, allowing the viral DNA to enter the host cytoplasm.At the distal end of the tail is a base plate, which functions as the attachment platform for tail fibers and tail spikes. The baseplate anchors the phage to the bacterial surface, while the tail fibers recognize and bind specific receptors on the host cell, thereby determining host specificity. Tail spikes may facilitate irreversible attachment and, in some phages, possess enzymatic activity that degrades bacterial surface polysaccharides to promote infection.The receptor-binding domains are located on specialized receptor-binding proteins (RBPs)present on the tail fibers or tail spikes. These proteins recognize specific receptors on the bacterial surface, including lipopolysaccharides (LPS), outer membrane proteins (e.g., OmpC and OmpF), teichoic acids, capsular polysaccharides, flagella, and type IV pili, depending on the bacterial host and phage species. This highly specific interaction between RBPs and bacterial receptors is the primary determinant of phage host range and is responsible for the exceptional specificity of bacteriophages as biological control agents (Latka *et al*. 2021; Pires *et al*. 2022; Turner *et al*. 2023).

Where are Bacteriophages Found?

Bacteriophages are the most abundant biological entities on Earth and are ubiquitously distributed in environments where their bacterial hosts occur. It is estimated that approximately 10^{31} phage particles exist in the biosphere, with marine ecosystems alone containing up to 10w phage particles per millilitre of seawater (Batinovic*et al*. 2019; Dion *et al*. 2020). Since bacteriophages replicate only within susceptible bacterial hosts, their distribution closely follows that of their host bacteria. Consequently, phages can be isolated from diverse sources, including soil, freshwater, seawater, wastewater, sewage, compost, irrigation water, rhizosphere and phyllosphere soils, infected plant tissues, and other agricultural environments (Farooq *et al*. 2022; Halawa *et al*. 2023).For plant pathogenic bacteria, bacteriophages are commonly recovered from infected plant tissues, crop rhizospheres, irrigation water, and nearby soil. For example, phages specific to *Xanthomonaseuvesicatoria* have been isolated from pepper rhizosphere soil, seeds, and irrigation water, while *Xanthomonasoryzae*pv. *oryzae* phages have been recovered from floodwater surrounding rice fields (Gašiæ*et al*.2011; Chae *et al*. 2014). In addition, wastewater and organic waste have also been identified as valuable reservoirs for isolating diverse bacteriophages (Carstens *et al*. 2019). In India, the Ganga River harbors a rich diversity of bacteriophages, contributing to its natural self-purification capacity.

Isolation of bacteriophages is relatively simple and typically involves enrichment of environmental samples with the target bacterial host, followed by plaque assays for phage detection, purification, and propagation. Enrichment is particularly useful when phages are present at low concentrations and significantly improves the efficiency of phage isolation (Pires *et al*. 2022; Turner *et al*. 2023).

How Bacteriophages Work

It is an important deciding factor determining whether a phage is applicable for biocontrol. Phages can be grouped into two based on their mechanism of action or replication: Lytic phages and lysogenic phages. Bacteriophages infect the bacteria by using lysogenic or lytic cycles (Jamal *et al*. 2019). In the case of the lysogenic cycle, bacteriophage and bacteria have guest and host relation. Here the bacteriophage integrates its genome directly into bacterial cell chromosomes after which they replicate and form daughter cells. They initiate the infection by attaching themselves to specific bacteria by adsorption and inserting their genome into the bacterial cytoplasm. In the case of the lytic cycle, bacteriophage and bacteria have master and slave relation. Here the phages are intended to utilize the ribosomes of host bacteria to make their proteins right after the injection of genetic material. At the end of this cycle, phage has produced some virion components that are lysin and holins. Lysins are

also known as endolysin that cause lysis of bacterial cells. The virulent nature of lytic phages along with their exponential increase in numbers after each infection cycle makes them the best alternative of pesticides use.

Lytic Cycle

1. Adsorption – in this process the attachment of phage to the surface of susceptible host bacterium takes place. Tips of tail fibers attach to specific receptor sites on the host bacterium. Host specificity of phage is determined in adsorption stage of cycle itself. Specific strains of bacteriophages can only adsorb to specific strain of host bacteria which is known as viral specificity. Infection of bacterium by naked phage nucleic acid is known as trans infection.

2. Penetration – this process resembles injection through a syringe. The tail sheath of the phage contracts after adsorption. Base plate and tail fibers attach firmly to the bacterial cell. Bacteriophage enzyme (holin) drills a hole in the bacterial wall to let endolysin access and break the cell wall and the hollow core is pushed downwards through it. Bacteriophage injects its genome inside the bacterial cell. After penetration empty head and tail of phage remain outside bacterial cell called ghosts/doughnut.

3. Synthesis/Replication – during replication virus uses host bacterium's metabolic machinery to produce viral structural components and viral enzymes. Replication is divided into three phases: early phase, middle phase and late phase. In case of early phase, after entering host cell, enzymes coded by bacteriophage genome shut down bacterium's protein, DNA, RNA synthesis. Then in middle phase, viral nucleic acid is produced separately in bacterial cell and then the viral genome is transcribed into viral mRNA. At last, in the late phase, viral mRNAs are translated into viral proteins (head and tail proteins) by host bacterium's ribosomes.

4. Maturation - on maturation, the head and tail protein of phage DNA get assembled. Each component of phage DNA is surrounded by a protein coat. Then the tail structures are added forming a virion.

5. Release – release of phages typically takes place by lysis of bacterial cell. During replication of phage, bacterial cell wall weakens and assumes spherical shape then a bacteriophage encoded lysozyme degrade bacterial cell wall and breaks down bacterial peptidoglycan. This causes lysis of bacterium and release of intact bacteriophages. At a time 100 to 200 phages are released and the entire lytic cycle from phage's first contact with cell surface to cell lysis takes only 20-30 minutes at 37!.

Lysogenic Cycle

1. Adsorption – in this process the attachment of phage to the surface of susceptible host bacterium takes place. Tips of tail fibers attach to specific receptor sites on the host bacterium. Host specificity of phage is determined in adsorption stage of cycle itself. Specific strains of bacteriophages can only adsorb to specific strain of host bacteria which is known as viral specificity. Infection of bacterium by naked phage nucleic acid is known as transinfection.

2. Penetration – this process resembles injection through a syringe. The tail sheath of the phage contracts after adsorption. Base plate and tail fibers attach firmly to the bacterial cell. Bacteriophage enzyme (holin) drills a hole in the bacterial wall and the hollow core is pushed downwards through it. Bacteriophage injects its genome inside the bacterial cell. After penetration empty
head and tail of phage remain outside bacterial cell called ghosts/doughnut.

3. Integration – in this process instead of killing host, phage DNA integrates into bacterial chromosomes and becomes part of it. This integrated state is referred as prophage, a key feature distinguishing lysogenic cycle from lytic cycle. Prophage remains inactive and does not produce viral particles. Bacteria carrying a prophage without being lysed is called lysogenic bacteria and the process is called lysogeny. Lysogenic state of virus may persist for an extended period.

4. Replication – as bacterium replicates its DNA, it makes numerous copies of the prophage.

As host cell undergoes cell division, viral genes subsequently pass on to the daughter cells and their DNA. Phage presence may alter phenotype of bacteria since it can bring in extra genes. This change in host phenotype is called lysogenic conversion/phage conversion.

5. Induction – this process gets involved under certain conditions; the phage is triggered to exit bacterial chromosome and initiate lytic cycle. This occurs in presence of environmental stresses like UV radiation or presence of specific chemical signals. The phage then synthesizes its own head and tail protein, gets matured into a virion and releases by the lysis of bacterial cell.
Lysogenic phages are generally unsuitable for biological control of plant pathogenic bacteria. Unlike lytic phages, they do not immediately lyse the host cell, resulting in slower pathogen suppression. Instead, they integrate their genome into the bacterial chromosome as a prophage, where they remain dormant until induced. During this stage, lysogenic phages may facilitate horizontal gene transfer through transduction, including the transfer of virulence or antibiotic resistance genes, potentially increasing bacterial pathogenicity. Furthermore, prophage-carrying bacteria often exhibit superinfection exclusion, preventing infection by closely related bacteriophages and thereby reducing the effectiveness of subsequent phage applications. For these reasons, strictly lytic bacteriophages are preferred for agricultural biocontrol(Hoffmann *et al*. 2025).

Application Strategies

Bacteriophages can be applied for the management of bacterial plant diseases either as monophage therapy or phage cocktail therapy, depending on the target pathogen and disease complexity (Farooq *et al*. 2022; Halawa *et al*. 2023).

Monophage therapy involves the use of a single lytic bacteriophage to infect and lyse a specific bacterial pathogen. Owing to its high host specificity, this approach is most suitable for genetically uniform bacterial populations and situations where a well-characterized phage is available. Monophage therapy preserves beneficial microorganisms and is relatively simple to characterize and formulate, and is commonly used in laboratory and greenhouse studies. However, its narrow host range and the rapid emergence of phage-resistant bacterial mutants often limit its effectiveness under field conditions (Pandit *et al*. 2022). Studies have also shown that filamentous phages can reduce bacterial virulence by altering the expression of pathogenicity-related genes. For example, öRSM3 infection reduced the virulence of *Ralstonia solanacearum*, while XacF1 infection impaired the pathogenicity of *Xanthomonas axonopodis* pv. *citri* (Addy *et al*. 2012; Ahmad *et al*. 2014).

In contrast, phage cocktail therapy, which combines two or more compatible lytic bacteriophages, is considered a more effective strategy for agricultural applications. By targeting multiple bacterial receptors or strains simultaneously, phage cocktails broaden the host range, enhance bacterial killing, and significantly reduce the likelihood of resistance development. They are particularly effective against genetically diverse pathogen populations and mixed infections, making them more suitable for field conditions. However, careful selection of compatible phages is essential to avoid antagonistic interactions, such as receptor competition or superinfection exclusion, and to ensure cocktail stability (Pires *et al*. 2022; Turner *et al*. 2023). Several studies have demonstrated the superior performance of phage cocktails over single-phage applications. Wang *et al*. (2019) reported that increasing the number of phages in *Ralstonia solanacearum* cocktail significantly reduced bacterial wilt severity in tomato, while Álvarez *et al*. (2019) observed greater disease suppression using two- or three-phage cocktails than with individual phages. Recent studies further confirm that optimized phage cocktails provide broader host coverage, improved field efficacy, and more durable disease control, highlighting their potential as sustainable tools for integrated management of bacterial plant diseases (Farooq *et al*. 2022; Halawa *et al*. 2023).

Phages and several compounds have been reported to inhibit biofilm formation in bacteria. This results in enhanced virulent phage efficacy.

*E. amylovora*phage L1 capsids produce the enzyme depolymerase that inhibits biofilm formation by degrading extracellular polysaccharide (EPS). Biofilm degradation allows the broad host-range *E. amylovora*phage Y2 to adsorb on bacterial cells for infection (Born *et al*.2017). Papaianni*et al*. (2020)observed a synergistic effect of long fatty acid (LFA) and phage in biofilm eradication of *X. campestris*pv. *campestris*. LFA mimics the diffusible signalling factor (DSF) to induce biofilm dispersal, which makes bacteria more susceptible to phage lysis.

Application methods

The efficacy of bacteriophage-based biocontrol largely depends on the method of application, which should maximize contact between phages and their target bacterial pathogens. The choice of application method depends on the pathogen's ecology, infection site, and crop type. Phages are commonly applied as liquid formulations prepared in sterile water or suitable buffers (e.g., SM buffer) at concentrations ranging from 10w –10y plaque-forming units (PFU) mL {¹. To improve persistence under field conditions, formulations may include protective additives or carrier microorganisms that enhance phage survival against environmental stresses such as ultraviolet radiation, desiccation, and temperature fluctuations (Buttimer *et al*. 2017; Farooq *et al*. 2022; Halawa *et al*. 2023).

The major methods of bacteriophage application include :

- **Foliar spray:** Phages are sprayed directly onto foliage to control leaf-infecting bacterial pathogens such as *Xanthomonas citri* and *Pseudomonas syringae*. This is the most widely adopted method for managing foliar bacterial diseases.

- **Stem injection or trunk infiltration:** Phages are introduced directly into the vascular tissues to target systemic pathogens such as *Xylella fastidiosa*, the causal agent of Pierce's disease of grapevine.

- **Soil drenching:** Phage suspensions are applied to the rhizosphere or root zone for the management of soil-borne pathogens, particularly *Ralstonia solanacearum*, which causes bacterial wilt.

- **Seed and seedling treatment:** Seeds or seedlings are immersed in phage suspensions before sowing or transplanting to prevent early infection by seed-borne bacterial pathogens, including *Xanthomonasoryzae*pv. *oryzae*, the causal agent of bacterial leaf blight of rice.

Commercial Formulations

The commercialization of bacteriophage-based biopesticides has advanced considerably over the past two decades. Among the pioneering companies, OmniLytics,Inc. (USA) developed AgriPhage®, the first bacteriophage-based biopesticide registered by the U.S. Environmental Protection Agency (EPA) for the management of bacterial spot caused by *Xanthomonas* spp. in tomato and pepper, and bacterial speck caused by *Pseudomonas syringae*pv. *tomato*. Since then, several phage-based products have been developed for the management of economically important bacterial diseases, demonstrating the growing commercial potential of bacteriophage technology in sustainable agriculture (Jones *et al*.2021; Farooq *et al*. 2022; Halawa *et al*.,2023).

Factors affecting the efficacy of Bacteriophages for Plant Disease Control

Initial Concentration of Bacteriophages

A minimum number of phages is required to effectively control bacteria. According to Kasman *et al*. (2002) the effective concentration of phages to kill bacteria depends upon host bacterial density and rate of adsorption. Balogh (2002) demonstrated that a threshold bacteriophage concentration must be present on the tomato phyllosphere to control bacterial spot of tomato disease pathogen *X. perforans*. They found that a minimum concentration of 10^6 PFU/ml was required to control bacterial leaf spot in the greenhouse and a higher concentration of 10^4 PFU/ml provided statistically similar results, while a concentration of 10^4 PFU/ml was ineffective.
Cabbage black rot bacteria (*X. campestris*pv. *campestris*) biofilm formation is greatly reduced

at 10^8 PFU/ml bacteriophage application, in comparison to 10^6 PFU/ml, due to disaggregation of the biofilm by galactose present in phage capsids (Papaianni*et al*. 2020). Adachi *et al*. (2012)found that rice seedling blight (*Burkholderiaplantarii*) incidence was lower at 10^7 PFU/ml in comparison to 10^6 PFU/ml in stagnant water conditions, but phage concentrations of 10^5-10^8 PFU/ml in circulating water had similar disease control. This indicates that constant agitation of water increases phage interaction with bacteria. Lang *et al*. (2007) also found no difference in *Xanthomonas* leaf blight of onion severity at 10^5-10^8 PFU/ml bacteriophage cocktail application.

Development of Resistance to Bacteriophages

Bacteria have a high probability of becoming resistant to phages, a problem since the early days of phage therapy. Bacteria develop resistance by mutation of an essentially required protein or modification of a phage adsorption receptor or an altruistic abortive infection (Abi), a complex mechanism in which phage infected cells commit suicide in order to prevent phage reproduction and provide bacterial host population immunity, similar to a hypersensitive response in plants. Another mechanism of resistance is based on bacteria utilizing the CRISPR/CAS system against foreign DNA. The latter system is present in 40% of bacteria (Burstein *et al*.2016). This system is also found in the plant pathogen *P. atrosepticumi*, *E.amylovora*and *X. oryzae*. Bacteria developing resistance to phages may also have a fitness cost due to a reduction in virulence, resulting in reduced disease severity. Mutation in type IV pili in *Xanthomonas* spp. and *Xylella fastidiosa* impart resistance to phages, but reduce the virulence of bacteria due to loss of twitching motility.

Strain Variation in Target Bacteria

Bacterial strains that cause plant diseases of various hosts have significant variation, which poses a serious concern for use of phages as a biocontrol agent. This problem is magnified by narrow host-range bacteriophages that only infect a particular strain of a bacterial species. For example, bacteria causing the bacterial leaf spot of tomato and pepper, formerly considered one bacterial species, *X. campestris*pv. *vesicatoria*, is now classified as four different species with each bacterial species consisting of highly diverse strains (Schwartz *et al*. 2015). For example, in a study by Bouzar *et al*. (1999), 120 *X. euvesicatoria* strains isolated from various countries in the Caribbean region were typed using 26 bacteriophages, and at least 26 different phage lysis patterns were identified. In another study, soft rot bacterial phages LIMEstone1and LIMEstone2 were specific to *Dickeyasolani*, while ÕD3 and ÕD5 were broad host-range phages to *D. solani*, *D. dianthicola*, *D. zeae*, *D. dadantii*, and *D. chrysanthemi* (Czajkowski*et al*. 2014).

Persistence of Bacteriophages in rhizospheres and phyllospheres

Maintaining sufficiently high phage populations on host surfaces for adequate time is essential for effective adsorption and control of phytopathogens; however, limited persistence is a major constraint in phage-based biocontrol. Reduction in phytopathogen density is directly proportional to the diversity and abundance of bacteriophages present in the phyllosphere or rhizosphere (Wang *et al*., 2019). In the phyllosphere, bacteriophage survival is severely affected by environmental factors, particularly ultraviolet (UV) radiation, along with temperature extremes, pH variation, desiccation, and wash-off due to rain. UV-B radiation causes DNA damage and is the most detrimental factor for phages exposed on plant canopies. Several studies have reported rapid phage inactivation under UV exposure, including complete elimination within hours or minutes depending on dose and exposure duration (Iriarte *et al*. 2007; Czajkowski *et al*. 2014; Born *et al*. 2015).

Compared to the phyllosphere, the rhizosphere provides a relatively more stable environment for phage persistence, though it presents its own constraints such as soil pH, moisture, temperature, organic matter, and soil type. Phage survival is optimal in neutral soils, while extreme pH values and low moisture reduce persistence (Choudhary *et al*. 2021).Clay soils may enhance survival under stress conditions but can also

immobilize phages through adsorption onto charged particles. Despite these limitations, numerous phages have been isolated from soil, and many are capable of persisting for several weeks in the rhizosphere (Vu and Oh, 2020).

Advantages

❑ Bacteriophages are naturally occurring biological agents that are abundant in diverse environments, making them readily available for isolation and application.

❑ They are biodegradable, non-toxic, and environmentally friendly, leaving no harmful chemical residues while preserving soil fertility and beneficial microbial communities.

❑ Bacteriophages are safe for plants, animals, and humans because they specifically infect bacterial cells and do not affect eukaryotic organisms.

❑ They are self-replicating in the presence of susceptible bacterial hosts, allowing their populations to increase naturally at the site of infection and reducing the need for repeated applications.

❑ Relatively low quantities of bacteriophages are often sufficient to suppress bacterial phytopathogens effectively due to their ability to multiply within the target host.

❑ Their high host specificity minimizes adverse effects on non-target and beneficial microorganisms, making them suitable components of integrated disease management programs.

❑ Bacteriophages are effective against bacterial strains that have developed resistance to copper-based bactericides and antibiotics.

❑ Many bacteriophages can penetrate and disrupt bacterial biofilms, improving the control of pathogens that are often difficult to manage using conventional chemical treatments.

❑ Bacteriophages are generally compatible with many chemical bactericides and biological crop protection products, facilitating their integration into existing disease management strategies.

Challenges

❑ The narrow host range of many bacteriophages limits their activity against genetically diverse bacterial populations. This limitation can be overcome by developing well-designed phage cocktail formulations.

❑ Phages are sensitive to environmental factors such as ultraviolet (UV) radiation, desiccation, temperature fluctuations, and extreme pH, which can reduce their persistence and efficacy under field conditions. Protective formulations, encapsulation technologies, and optimized application timing can improve their stability.

❑ Lysogenic bacteriophages may mediate horizontal gene transfer, including the transfer of virulence or other undesirable genes. Therefore, strictly lytic bacteriophages are preferred for agricultural applications.

❑ The absence of harmonized regulatory frameworks and clear registration guidelines in many countries, including India, has slowed the commercialization and adoption of phage-based biopesticides.

❑ Commercial availability of bacteriophage products remains limited, particularly in developing countries, restricting their widespread agricultural use.

❑ Large-scale production, formulation, storage, and quality control of bacteriophages require standardized manufacturing processes, which can increase production costs and affect commercial feasibility.

❑ Bacterial pathogens may develop resistance to individual bacteriophages. However, this challenge can be mitigated by using phage cocktails, periodically updating phage formulations, and integrating bacteriophages with other disease management practices.

CONCLUSION

Bacteriophages have emerged as promising biological control agents for the sustainable management of bacterial plant diseases, offering high host specificity, environmental safety, and effectiveness against antibiotic- and copper-resistant phytopathogens. Accumulating evidence indicates that lytic bacteriophages, particularly when formulated as phage cocktails, provide broader host coverage, reduce the emergence of phage-resistant bacterial populations, and improve disease suppression under diverse conditions. Recent advances in phage formulation technologies, engineered phages, and commercially available phage products further demonstrate the growing potential of bacteriophage-based biocontrol as a practical component of integrated plant disease management.

Despite these advances, several important knowledge gaps remain. Most available studies are confined to laboratory and greenhouse conditions, whereas long-term, multi-location field evaluations under diverse agro-climatic conditions are still limited. Standardized guidelines for phage isolation, characterization, cocktail formulation, dosage optimization, application timing, and quality control are lacking, making comparisons among studies difficult. The long-term ecological effects of repeated phage application on native microbial communities and the evolution of bacterial resistance under field conditions also remain insufficiently understood. Furthermore, greater emphasis is needed on developing broad-host-range and region-specific phage cocktails, improving formulation technologies to enhance environmental persistence, and integrating bacteriophages with other biological and cultural management practices. Regulatory harmonization, cost-effective large-scale production, and commercialization, particularly in developing countries, also require significant attention.

Addressing these gaps through interdisciplinary research will improve the consistency, reliability, and adoption of bacteriophage-based technologies, enabling them to become an effective and sustainable alternative to conventional bactericides for the long-term management of bacterial plant diseases.

REFERENCES

Adachi, N.,Tsukamoto, S., Inoue, Y., Azegami, K. 2012. Control of bacterial seedling rot and seedling blight of rice by bacteriophage.*Plant Dis*.**96**: 1033–1036.

Addy, H.S.,Askora, A., Kawasaki, T., Fujie, M., Yamada, T.2012. Loss of virulence of the phytopathogen *Ralstonia solanacearum* through infection ÕRSM filamentous phages. *Phytopathology***102**: 469-477.

Ahmad, A.A.,Askora, A., Kawasaki, T., Fujie, M., Yamada, T.2014. The filamentous phage XacF1 causes loss of virulence in *Xanthomonas axonopodis*pv. *citri*, the causative agent of citrus canker disease. *Front.Microbiol*. **5**: 321.

Álvarez, B.,López, M.M., Biosca, E.G. 2019. Biocontrol of the major plant pathogen *Ralstoniasolanacearum* in irrigation water and host plants by novel waterborne lytic bacteriophages. *Front.Microbiol*. **10**: 2813.

Balogh, B. 2002. Strategies for improving the efficacy of bacteriophages for controlling bacterial spot of tomato. M.S. Thesis, University of Florida, Gainesville, Florida, USA, 74 pp.

Batinovic, S.,Wassef, F., Knowler, S.A., Rice, D.T.F., Stanton, C.R., Rose, J., Tucci, J., Nittami, T., Vinh, A., Drummond, G.R., Sobey, C.G., Chan, H.T., Seviour, R.J., Petrovski, S. Franks, A.E. 2019. Bacteriophages in natural and artificial environments. *Pathogens***8**: 3-12.

Born, Y.,Bosshard, L., Duffy, B., Loessner, M.J., Fieseler, L. 2015. Protection of *Erwinia amylovora* bacteriophage Y2 from UV-induced damage by natural compounds. *Bacteriophage***5**: e1074330.

Born, Y.,Fieseler, L., Thöny, V., Leimer, N., Duffy, B., Loessner, M.J. 2017. Engineering of bacteriophages Y2::dpoL1-C and Y2::luxAB for efficient control and rapid detection of the fire blight pathogen, *Erwinia amylovora*. *Appl. Environ.Microbiol*. **83**: e00341-17.

Bouzar, H.,Jones, J.B., Stall, R.E., Louws, F.J., Schneider, M., Rademaker, J.L., de Bruijn, F.J. and Jackson, L.E. 1999. Multiphasic analysis of Xanthomonads causing bacterial spot disease on tomato and pepper in the Caribbean and Central America: evidence for common lineages within and between countries, *Phytopathology***89**: 328–335.

Burstein, D.,Sun, C.L., Brown, C.T., Sharon, I., Anantharaman, K., Probst, A.J., Thomas, B.C., Banfield, J.F.2016. Major bacterial lineages are essentially devoid of CRISPR-Cas viral defence systems, *Nature Commun*.**7**: 10613.

Buttimer, C.,McAuliffe, O., Ross, R.P., Hill, C., O'Mahony, J.,Coffey, A. 2017. Bacteriophages and bacterial plant diseases. *Front.Microbiol*. **8**: 34.

Carstens, A.B.,Djurhuus, A.M., Kot, W., Hansen, L.H. 2019. A novel six-phage cocktail reduces *Pectobacteriumatrosepticum* soft rot infection in potato tubers under simulated storage conditions. *FEMS Microbiol. Letters*. 366: fnz101.

Chae, J.C.,Nguyen, B.H., Yu, S.M., Lee, H.K., Lee, Y.H.2014. Diversity of bacteriophages infecting *Xanthomonas oryzae*pv. *oryzae* in paddy fields and its potential to control bacterial leaf blight of rice. *J. Microbiol.Biotechnol*. **24**: 740-747.

Choudhary, M.,Paret, M., Obradoviæ, A., Gašiæ, K., Jones, J.B. 2021. Bacteriophages to control plant diseases. In: *Microbial bioprotectants for plant disease management* (Eds. Köhl, J. and Ravensberg, W.J.), Burleigh Dodds Science Publishing, Cambridge, UK, pp. 473-506.

Czajkowski, R.,Ozymko, Z., Lojkowska, E. 2014. Isolation and characterization of novel soilborne lytic bacteriophages infecting *Dickeya* spp. biovar 3 ('*D. solani*'). *Plant Pathology*. **63**: 758-772.

Dion, M.B.,Oechslin, F., Moineau, S. 2020. Phage diversity, genomics and phylogeny. *Nature Reviews Microbiology*.**18**: 125–138.

Elnahal, A.S.M.,El-Saadony, M.T., Saad, A.M., Desoky, E.S.M., El-Tahan, A.M., Rady, M.M.,AbuQamar, S.F., El-Tarabily, K.A. 2022. Biological control agents as sustainable alternatives for plant disease management. *Plants***11**: 3190.

FAO and WHO. 2019. The FAO Action Plan on Antimicrobial Resistance 2016–2020. Food and Agriculture Organization of the United Nations and World Health Organization, Rome, Italy.

Farooq, T., Hussain, M.D., Shakeel, M.T., Tariqjaveed, M., Aslam, M.N., Naqvi, S.A.H., Amjad, R., Tang, Y., She, X., He, Zifu. 2022. Deploying viruses against phytobacteria: potential use of phage cocktails as a multifaceted approach to combat resistant bacterial plant pathogens. *Viruses***14**: 2-18.

Gašiæ, K., Ivanoviæ, M.M., Ignjatov, M., Caliæ, A. and Obradoviæ, A.2011. Isolation and characterization of *Xanthomonas euvesicatoria* bacteriophages. *J. Plant Pathol*.**93**: 415-423.

Halawa, E.M. 2023. Challenges of bacteriophages application in controlling bacterial plant diseases and how to overcome them. *J. Genet.Engineer.Biotechnol*.**21**: 20-25.

Hoffmann, A.,Sadowska, K., Zenelt, W., Krawczyk, K. 2025. Bacteriophages as a sustainable tool for plant disease management: benefits and challenges. *Agronomy*.**15**: 2507.

International Committee on Taxonomy of Viruses (ICTV). 2024. *Virus Taxonomy: 2024 Release.*International Committee on Taxonomy of Viruses.

Iriarte, F.B.,Balogh, B., Momol, M.T., Smith, L.M., Wilson, M., Jones, J.B. 2007. Factors affecting survival of bacteriophage on tomato leaf surfaces. *Appl. Environ.Microbiol*.**73**: 1704-1711.

Jamal, M.,Bukhari, S.M., Andleeb, S., Ali, M., Raza, S., Nawaz, M. Hussain, T., Rahman, S., Shah, S.S.A. 2019. Bacteriophages: an overview of the control strategies against multiple bacterial infections in different fields. *J. Basic Microbiol*. **59**: 123-133.

Jones, J.B.,Svircev, A.M., Obradoviæ, A.Ž. 2021. Bacteriophages for plant disease control. *Annual Rev.Phytopathol*.**59**: 155-177.

Kasman, L.M.,Kasman, A., Westwater, C., Dolan, J., Schmidt, M.G., Norris, J.S.2002. Overcoming the phage replication threshold: a mathematical model with implications for phage therapy. *J.Virol*. **76**: 5557-5564.

Lamichhane, J.R.,Osdaghi, E., Behlau, F., Köhl, J., Jones, J.B., Aubertot, J. N. 2018. Thirteen decades of antimicrobial copper compounds applied in agriculture: A review. *Agron. for Sustain. Develop*.**38**: 28.

Lang, J.M.,Gent, D.H., Schwartz, H.F. 2007. Management of Xanthomonas leaf blight of onion with bacteriophages and a plant activator, *Plant Dis*.**91**: 871-878.

Leitner, L.,Ujmajuridze, A., Chanishvili, N., Goderdzishvili, M., Chkonia, I., Rigvava, S., Chkhotua, A., Changashvili, G., McCallin, S., Schneider, M.P., Liechti, M.D., Mehnert, U., Bachmann, L.M., Sybesma, W. and Kessler, T.M. 2021. History and therapeutic potential of bacteriophages. *Viruses***13**: 2106.

Mansfield, J.,Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronal, P., Dow, M., Verdier, V., Beer, S.V., Machado, M.A., Toth, I., Salmond, G. and Foster, G.D. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. *Molec. Plant Pathol.***13**: 614–629.

McManus, P.S.,Stockwell, V.O., Sundin, G.W., Jones, A.L. 2002. Antibiotic use in plant agriculture. *Annu. Rev.Phytopathol.***40**: 443–465.

Nakayinga, R.,Makumi, A., Tumuhaise, V., Tinzaara, W. 2021.*Xanthomonas* bacteriophages: A review of their biology and biocontrol applications in agriculture. *BMC Microbiol*. **21**: 291.

Pandit, R.,Kumar, J., Gulati, S., Bhandari, N., Mehta, P., Katyal, R., Rawat, C.D., Mishra, V., Kaur, J. 2022. Bacteriophages as biocontrol agents for plant bacterial diseases. *Plants*. **11**: 1975.

Papaianni, M.,Paris, D., Woo, S.L., Fulgione, A., Rigano, M.M., Parrilli, E., Tutino, M.L., Marra, R., Manganiello, G., Casillo, A., Limone, A., Zoina, A., Motta, A., Lorito, M., Capparelli, R. 2020. The union is strength: the synergic action of long fatty acids and a bacteriophage against *Xanthomonas campestris* biofilm. *Microorganisms* **9**: 60.

Ryan, R.P., Vorhölter, F.J., Potnis, N., Jones, J.B., Van Sluys, M.A., Bogdanove, A.J., Dow, J.M. 2011. Pathogenomics of *Xanthomonas*: understanding bacterium–plant interactions. *Nature Rev.Microbiol.* **9**: 344–355.

Schwartz, A.R.,Potnis, N., Timilsina, S., Wilson, M., Patané, J., Martins Jr, J., Minsavage, G.V., Dahlbeck, D., Akhunova, A., Almeida, N., Vallad, G.E., Barak, J.D., White, F.F., Miller, S.A., Ritchie, D., Goss, E., Bart, R.S., Setubal, J.C., Jones, J.B. and Staskawicz, B.J. 2015. Phylogenomics of *Xanthomonas* field strains infecting pepper and tomato reveals diversity in effector repertoires and identifies determinants of host specificity. *Front.Microbiol*. **6**: 535.

Sundin, G. W., Wang, N. 2018. Antibiotic resistance in plant-pathogenic bacteria. *Annu. Rev.Phytopathol*.**56**: 161–180.

Turner, D.,Shkoporov, A.N., Lood, C., Millard, A.D., Dutilh, B.E., Alfenas-Zerbini, P. Zyl, L.J.V., Aziz, R.K., Oksanen, H.M., Poranen, M.M., Kropinsk, A.M., Barylski, J., Brister, J.R., Chanisvili, N., Edwards, R.A., Enault, F., Gillis, A., Knezevic, P., Krupovic, M., Kurtboke, I., Kushkina, A., Lavigne, R., Lehman, S., Lobocka, M. and Adriaenssens, E.M. 2023. Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee. *Arch.Virol.* **168**: 74

Vu, N.T. and Oh, C.S. 2020. Bacteriophage usage for bacterial disease management and diagnosis in plants. *Plant Pathol. J*.**36**: 204–217.

Wang, X.,Wei, Z., Yang, K., Wang, J., Jousset, A., Xu, Y., Shen, Q. and Friman, V.P. 2019. Phage combination therapies for bacterial wilt disease in tomato. *Nature Biotechnol*. **37**: 1513-1520.