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Harnessing phages as biocontrol agents against common bacterial blight diseases in plants

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Abstract

Bacterial blight diseases in plants are caused by various bacterial pathogens such as *Dickeya, Pectobacterium*, *Xanthomonas*, and others. They infect a wide range of crops including potatoes, tomatoes, apples, and citrus. The impact of these diseases includes reduced crop yields, lower quality produce, and economic losses for farmers. Traditional control methods like antibiotics and copper-based compounds are becoming less effective due to the development of resistance and environmental concerns. As a result, there is growing interest in alternative strategies such as biocontrol agents (BCAs), particularly bacteriophages. Bacteriophages are viruses that infect bacteria, they offer a promising solution to agricultural disease management. Their specificity and ability to target pathogenic bacteria make them an environmentally friendly alternative to chemical interventions. By optimizing phage formulations and application protocols, bacteriophages have the potential to contribute to healthier crops, increased yields, and a more sustainable agricultural system.

Keywords: Phages; Bacterial blight diseases; Biocontrol agents; Resistance; Phytopathogens

1. Introduction

The world's population is expected to reach approximately 9.6 billion by 2050, leading to potential scarcities in agricultural and food resources. To address this challenge, agricultural efficiency must be improved, with crop production projected to increase by 70–80% (1). However, various factors such as climate change, technological gaps, pests, and plant diseases hinder production rates (2, 3). Plant diseases particularly impact around 10% of global food in developing and emerging nations. These diseases are caused by various pathogens including parasitic plants, fungi, viruses, nematodes, and bacteria, collectively known as phytopathogens (4). Among bacterial phytopathogens, approximately 200 species have been identified, with notable genera including *Xanthomonas, Ralstonia, Erwinia*, *Pseudomonas,* and *Pectobacterium*. These pathogens exhibit high virulence, adaptability to changing environments, and pose significant challenges for management. *Dickeya and Pectobacterium*, both belonging to the family Enterobacteriaceae, collectively known as the Soft Rot Enterobacteriaceae (SRE), pose significant threats to a wide range of plant hosts. These genera produce cell-wall-degrading enzymes enabling them to infiltrate and macerate plant tissue. The economic impact of infections caused by these bacteria, particularly on crops like potato, can be severe, resulting in substantial annual losses in various regions worldwide (5, 6, 7, 8). *Erwinia amylovora*, responsible for fire blight, affects species within the Rosaceae family, notably apple and pear trees. The disease has a global presence and significant economic consequences, necessitating stringent quarantine measures in affected regions. Traditional control

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methods involve cultural practices and preventative sprays, although concerns regarding antibiotic resistance highlight the need for alternative control strategies (9, 10, 11, 12). *Ralstonia solanacearum*, a Gram-negative soil-borne bacterium, poses a significant threat to over 200 plant species across various families, causing diseases like bacterial wilt and brown rot. Control strategies primarily involve cultural practices and the use of resistant cultivars, albeit with challenges such as negative correlations between resistance and yields (13, 14). *Pseudomonas syringae*, a diverse bacterial phytopathogen, exhibits a broad host range and causes diseases like bacterial speck in tomatoes. Control measures include the use of uncontaminated seeds and bactericides, although the emergence of copper-tolerant strains poses challenges to effective management (15, 16, 17). *Xanthomonas species*, with their high degree of host specificity, infect numerous important crops like tomato and pepper, causing diseases such as bacterial spot. Control methods involve cultural practices, resistant cultivars, and chemical controls, although challenges such as bacterial resistance to control agents persist (18, 19). *Xylella fastidiosa*, a xylem-limited phytopathogen, poses a significant threat to crops like grapevines, citrus, and olive trees. Disease management strategies include the removal of infected plants and control of insect vectors, albeit with challenges related to the use of insecticides and their impact on non-target organisms (20, 21, 22, 23). Understanding the characteristics and impact of common bacterial blight diseases is crucial for implementing effective management strategies and safeguarding agricultural productivity globally.

Efficient disease management is crucial for ensuring a stable and productive food supply. While antibiotics and copper-based compounds have been widely used to control phytopathogenic bacteria, they come with associated risks such as the emergence of resistant species and environmental harm (24,25, 26). Concerns over their toxicity have led to bans on these compounds in many western countries (27). Given these drawbacks, there is an urgent need for alternative plant disease control agents that offer effective protection with minimal environmental impact (28). Biocontrol agents (BCAs) represent a promising alternative, harnessing the power of microorganisms to combat plant pathogens (29). Biological control involves using living organisms to suppress the activities and reproduction of pathogenic bacteria. BCAs work by directly or indirectly manipulating microbial populations to reduce disease incidence (30). This approach offers a sustainable solution to disease management, mitigating the need for harmful chemical interventions while promoting environmental health and crop productivity. The use of bacteriophages as biocontrol agents (BCAs) has garnered significant interest in combating phytopathogens (31). Bacteriophages, viruses that exclusively infect bacteria, pose no harm to plants or animals. Discovered in the twentieth century, bacteriophages are considered abundant and diverse in the natural environment, playing vital roles in bacterial mortality and genetic exchange (32). They are ubiquitous, found across various domains and habitats, particularly in soil and oceans. Bacteriophages infect bacteria through lysogenic or lytic cycles (33). In the lysogenic cycle, a bacteriophage integrates its genome into bacterial cell chromosomes, leading to replication and the formation of daughter cells (34). In contrast, the lytic cycle involves bacteriophages attaching to specific bacteria, injecting their genome into the bacterial cytoplasm. Subsequently, they utilize the host bacteria's ribosomes to produce their proteins. The lytic cycle culminates in the production of virion components, including lysins, also known as endolysins, which cause lysis of bacterial cells (35). The virulent nature of lytic phages, coupled with their exponential increase in numbers after each infection cycle, positions them as a promising alternative to pesticide use (36).

Discovered in 1920, bacteriophages were soon recognized for their potential therapeutic applications in the agricultural sector. Many plant diseases caused by bacterial pathogens like *Xanthomonas campestris, Pseudomonas syringae, Dickeya solani,* and *Erwinia amylovora* have been reported to be cured by bacteriophages (37). Through targeted phage therapy, these pathogens can be effectively targeted, offering a sustainable and environmentally friendly approach to disease management in agriculture. The application of bacteriophages in agriculture represents a promising frontier in crop protection. By harnessing the natural predatory abilities of these viruses, farmers can mitigate the impact of bacterial diseases while reducing reliance on chemical interventions. Moreover, phage-based solutions have the potential to minimize collateral damage to beneficial organisms and ecosystems, thus promoting ecological balance in agricultural landscapes. As research into phage therapy continues to advance, there is growing optimism regarding its widespread adoption in agricultural practices. However, further studies are needed to optimize phage formulations, delivery methods, and application protocols to ensure efficacy and scalability in diverse agricultural settings. By leveraging the innate ability of phages to target specific bacterial pathogens, farmers can work towards healthier crops, increased yields, and a more environmentally sustainable agricultural system.

In this study, several key aspects regarding bacteriophages will be examined, including their historical significance, life cycle dynamics, limitations, advantages, and applications as biocontrol agents against bacterial blight diseases in agriculture.

2. Understanding common bacterial blight diseases

According to Britannica (38), blight is any of various plant diseases whose symptoms appear on the shoots as sudden and severe yellowing, browning, spotting, wilting or dying of leaves, stems, flowers and fruits or the entire plants. It is mostly caused either by bacterial or fungal infections referred to as bacterial blight or fungal blight respectively. Bacterial blight is caused by various species of bacteria mainly in the genus *Pseudomonas* and *Xanthomonas*. It affects several important economic crops worldwide leading to reduced yields or total crop loss (38). In Nigeria, several crops are affected by the disease (Table 1) with that occurring in rice caused by two different pathogens (39, 40).

Bacterial blight symptoms vary depending on the host plant and the specific bacterial species. It usually begins as small, water-soaked yellow spots. The spots enlarges or merges as the tissues in the center dies turning brown surrounded by yellowish-green ring (38). Transmission can occur through insect vectors, infected stems, fallen leaves, soil, rain-splash, and planting materials (41). Thus, management options must be tailored toward mitigating these means of introduction and spread. Cultural, chemical treatments and deployment of tolerant or resistant varieties in an integrated disease management system are crucial to minimizing bacterial blight's impact on agriculture (42, 43, 44, 45)

Cultural practices include methods such as intercropping, crop rotation, fallowing, farm sanitation, weed control, timely planting and use of clean planting materials which aim to reduce the pathogen inoculum sources over time (46). However, farmers often regard some of the recommended cultural method cumbersome to practice as a result of non-availability of land space and increased cost (47). Treatment of plants with copper-based fungicides and antibiotics such as streptomycin, oxytetracycline and kasugamycin has been recommended to prevent the disease (42, 44, 45). Availability, health implication, resistance development and cost are some of the limitations to the adoption by farmers.

Deployment of tolerant or resistant varieties is considered the main solution for control especially for resource poor farmers (48). Long cycle of resistant development, breakdown and seed availability have not assisted the farmers to utilize fully this option. Thus, other advance strategies for managing bacterial blight need to be explored, one of which is the use of Bacteriophages.

Bacteriophages, commonly known as phages, are viruses that infect and replicate within bacteria. They are being explored, because of specificity to their host, as a potential biological control method for bacterial diseases in plants, including bacterial blight. Phages do not affect human cells, making them a safe option for managing bacterial diseases in crops. The use of phages in agriculture offers a green and efficient strategy to control pathogens without the overuse of antibiotics, which is crucial for sustainable farming practices (49).

Research in Nigeria has shown that phages can effectively reduce bacterial blight incidence and severity as can be seen in Table 1 below:

Table 1: Bacterial blight reported in Nigeria

Disease Causal organism	Year of introduction	Symptom	Source
Cassava Bacterial blight	1972		[50, 51, 41, 46, 47]
Rice leaf blight (*Xanthomonas oryzae* pv. *oryzae*)	2009	Water-soaked lesions, yellowing, wilting.	[39]
Rice leaf blight (*Sphingomonas* sp)	2017	Symptoms included yellow-brown discolourations along one of the two leaf blades, turning brown to dark-brown with age. Severely affected leaves developed necrotic patches and died.	[40]
Soybean (*Pseudomonas syringae* pv.*glycinea*)	2006	Vein-limited water-soaked lesions on leaves with/without chlorotic halo and water-soaked lesions and misshaped fruits	[52]
Beans (*Xanthomonas axonopodis* pv. *phaseoli*)		Water-soaked spots on leaves, wilting, and pod rot.	[50, 41]

Cowpea (*Xanthomonas axonopodis* pv. *vignicola)*	1975		[53, 48]
Cotton (*Xanthomonas campestris* pv. *malvacearum*)	1972	Symptoms: Angular leaf spots, defoliation and boll rot.	[54, 55]

3. Phages- nature's bacterial assasins

Phages, specific viruses of bacteria, manipulate the metabolism of their bacterial hosts to facilitate replication. The majority of identified phages belong to the tailed phages, constituting the Taxonomic Order: Caudovirales (56). These phages feature icosahedral heads containing double-stranded DNA genomes. Within the Caudovirales order, three phage families exist: Myoviridae, characterized by rigid contractile tails; Podoviridae, possessing short, non-contractile tails; and Siphoviridae, distinguished by long flexible tails. Other phage families exhibit highly variable morphologies and genomes with diverse nucleic acid compositions. Bacteriophages were discovered over a century ago and are estimated to be the most diverse and abundant biological entities on Earth. They play crucial roles in controlling bacterial communities, nutrient cycling, and bacterial genome evolution (57, 58, 59, 60). While billions of bacteriophages infect various bacterial classes in the environment, the majority of characterized bacteriophages belong to the Caudovirales, exhibiting either lytic or lysogenic infection types (61). The figure below shows the infection cycle of phages.

Adsorption of bacteriophages

↓

Injection of genomic DNA (gDNA) into host cell cytoplasm

↓

Utilization of host bacterium ribosomes for phage protein synthesis

↓

Bacterium provides resources for phage genome replication and protein production

↓

Late stage: Bacteriophages encode holins and lysins (endolysins) to lyse the bacterium

↓

Release of phage progenies

↓

Integration of bacteriophage genome into bacterial cell chromosome (prophage)

↓

Replication and transfer to daughter bacterial cell

↓

Spontaneous alteration to lytic cycle under certain conditions

↓

Use of lytic bacteriophages for disease management

↓

Use of temperate bacteriophages for bacterial-pathogen diagnosis

↓

Detection of bacterial pathogens with phage proteins, including green fluorescent protein

Figure 1 Infection cycle of Bacteriophages

The infection cycle of bacteriophages initiates with the adsorption of the phages to specific receptors on the surface of susceptible bacteria. Upon irreversible attachment, the phages inject their genomic DNA (gDNA) into the cytoplasm of the host cell (61). In the lytic replication cycle, following gDNA injection, the phages utilize the host bacterium's ribosomes to synthesize phage proteins. The bacterium also provides resources for bacteriophage genome replication and the production of virion-related protein components (61). In the late stage of the lytic replication cycle, the phages encode holins and lysins, also known as endolysins, which ultimately lyse the bacterium, releasing the phage progenies (61). In contrast, the lysogenic replication cycle involves the integration of the bacteriophage genome into the bacterial cell chromosome, forming a prophage, or existing as an episomal element. In this state, the prophages replicate along with the bacterial chromosome and can transfer to daughter bacterial cells. Prophages have the potential to spontaneously switch to a lytic cycle, resulting in the lysis of the host bacterium (62). This switch can occur due to various environmental stresses, the metabolic condition of the host bacteria, or antibiotic treatment (63, 64). In general, lytic bacteriophages are typically employed in the management of bacterial diseases due to their ability to directly eliminate pathogens, thereby safeguarding plants against these harmful agents. Conversely, temperate bacteriophages may find utility in diagnosing bacterial pathogens owing to their integration with the bacterial chromosome. By incorporating various fusion proteins, such as green fluorescent protein, into lytic bacteriophages, bacterial pathogens carrying phage proteins can be readily detected. This facilitates efficient detection and monitoring of bacterial infections, aiding in timely disease management strategies.

4. **Phages vs. Bacterial blight: how it works:**

Bacteriophages, discovered by Frederick Twort and Felix d'Herelle in 1917, marked a significant breakthrough in microbiology. Their pioneering work suggested the existence of these viral entities, laying the foundation for subsequent research into their therapeutic potential (65). In 1919, d'Herelle reported the therapeutic efficacy of bacteriophages in treating patients with diarrhea, demonstrating their potential in combating bacterial infections in humans (66). Building upon these findings, numerous scientific studies explored the use of bacteriophages in treating various bacterial infections, including cholera and staphylococcal infections (67). The application of phages extended beyond human health to include plants and crops. In 1924, studies demonstrated the effectiveness of cabbage filtrate in inhibiting the growth of *Xanthomonas campestris* on cabbage crops, highlighting the potential of phages in plant disease management (68). Similarly, in 1925, researchers successfully controlled soft rot diseases on carrots and potatoes through bioassays (69). The first successful field trial of bacteriophage occurred in 1935, targeting Stewart's wilt disease caused by *Pantoea stewartia.* This milestone demonstrated the practical application of bacteriophages in treating plant diseases (70). Subsequent research efforts focused on combating highly virulent strains, such as *Agrobacterium tumefaciens,* leading to significant inhibition of bacterial activity (71). These early successes paved the way for further exploration and development of bacteriophage-based therapies, showcasing their promise in addressing bacterial infections across various domains, from human health to agricultural settings.

In the last decade, several bacterial plant pathogens were described by numerous scientists and pathologists. Out of them, one of the most important plant pathogens is the *P. syringae* responsible for causing several plant diseases in monocots as well as in dicot plants from all over the world (72). To check the efficacy of phage in this case, two different field trials were conducted on a phage cocktail of about six isolated bacteriophages. This trial was carried out by using a phage as a biocontrol against *P. syringae* that caused the bacterial blight disease in leek. So, it was evident from the experiment that the phage cocktail has the potential therapeutic cure for the bacterial blight disease and successfully eradicates the plant infection caused by the *P. syringe* (73, 74). Bacteriophages that are used for biocontrol in agriculture must be stable enough in the environment to tolerate (UV) radiations, temperature fluctuations, and chemical agents and must be lytic [2, 3]. Agriphage is a phage-based product, produced by the approved US Company Omnilytics, which can control the bacterial spot diseases of peppers and tomatoes. Several bacteriophage enzymes such as ΦXo411 and Lys411 have been isolated showing lytic activity against the *Xanthomonas* (75). Phage should be applied directly while treating bacterial infections at early stages for better and more efficient results. Referring to experimental trials, *Xanthomonas campestris* pv. *pruni* is a bacterial pathogen that causes leaf spots. It was subjected to two different treatment approaches. In the initial phase, the treatment was administered one hour prior to the bacterial inoculations, while in the subsequent phase, it was applied 24h in advance of the bacterial inoculation (76). The results were quite astonishing during the first trial.

Recently, sensitivity tests have been conducted to assess phage survival in various climatic conditions, both controlled and uncontrolled. When fruit was treated with a phage suspension, 92% of tested fruits remained disease-free. However, there was a tenfold decrease in phage population compared to that applied in controlled climatic chambers, attributed to adverse environmental conditions such as dehydration, UV radiation, and high temperatures (77). Researchers have identified optimal times for phage application, with dawn and dusk being the most effective. During these times, reduced UV radiation enhances phage activity, leading to improved efficacy (78). Selecting suitable bacteriophages for specific growing conditions is also crucial. For instance, phage RSL1, targeting *Ralstonia solanacearum*, exhibited significant resistance to high temperatures (37–50 °C). In tomato plants infected with *R. solanacearum*, phage RSL1 prevented wilting symptoms, highlighting its potential for disease management (79). In recent studies, potatoes and tomatoes have emerged as key crops benefiting from phage biocontrol applications. In European countries, a phage formulation targeting *Dickeya solani* has shown promise. In field trials, tubers inoculated with both the phage and bacterium exhibited only 10% tissue maceration, compared to 40% in control samples (80). *Streptomyces scabies* is a Gram-positive bacterium responsible for causing infections on potatoes leading to the formation of corky lesions generally known as common scabs and results in reduced growth of seedlings. The pathogen can be effectively treated by using phage, so the chances of infection in potato crop can be successfully eliminated. In summary, the above studies demonstrate phage potential in treating various plant diseases (81).

Bacteriophages have also been used in field conditions and greenhouses. To control *R. solanacearum*, phage was applied directly into the rhizosphere through soil drenching that was effective in the suppression of the development of wilting in the tomato plant (82). In the case of other soil-borne pathogens like *Xanthomonas euvesicatoria, X. campestris* pv. *campestris,* and *P. carotovorum* subsp*. carotovorum,* foliar spraying method was used to eradicate the disease incidence in plants caused by these pathogens. Furthermore, another application regarding the potential of filamentous phage has also been reported. The filamentous ΦRSM-type phage enhances the eradication of *R. solanacearum* in tomato plants (83).

Besides biocontrol applications, some significant factors affect the phage effectiveness used in the field of agriculture. In a recent evident study, it was demonstrated that the pH of the fruit has a vital impact on the phage stability as well as its activity (84). In studies involving fresh-cut melons, the application of a specific phage cocktail targeting Salmonella proved effective, resulting in a significant reduction in bacterial population by 3.5 logs at 10 °C and 2.5 logs at 20 °C. However, when the same phage cocktail was applied to apples, no reduction in bacterial population was observed, possibly due to the low pH of apples (85).

To broaden the host range of phages and mitigate the limitations of single phage treatments, researchers have developed bacteriophage cocktails, combining multiple phages into a single mixture. This approach not only compensates for host range limitations but also reduces the likelihood of bacteria developing resistance to phages (86). Understanding the effectiveness of phage cocktails against pathogens like *Xanthomonas* is crucial for assessing their performance under various environmental conditions (87). For instance, a recent study demonstrated the biocontrol activity of phages against *Pseudomonas syringae*. By aerosolizing a single phage or a phage cocktail containing 5% sucrose and 3% corn flour onto bean leaves prior to *P. syringae* inoculation, researchers observed a 60% reduction in disease severity with a single phage and a 70% reduction with the phage cocktail (88).

Bacteriophages have evolved numerous strategies to release hundreds of new progenies from the host to the external environment (89). Most filamentous phages constantly release new virions from the host cell through extracellular vesicles without lysing the bacterium. In some cases, bacteriophages release their progenies through lytic enzymes, which are quite beneficial in controlling pathogenic bacteria due to the phages' wide range of hosts. Bacteriophages have evolved group of lytic enzymes that are responsible for the degradation of the peptidoglycan layer of the bacterial host. These enzymes are known as endolysin [81]. During the late stages of replication, these hydrolases are activated with the help of holin proteins and destroy the peptidoglycan (PG) layer of the bacterial host which results in the release of progeny virions. Endolysins are classified into five different groups based on the bonds in peptidoglycan they target. Phage infecting Gram-negative bacteria produces endolysin with a single globular domain known as enzymatically active domain EAD that is responsible for the digestion of the PG layer (90).

Endolysins CN77 and CMP1 have also been reported for lysing *Clavibacter nebraskensis* and *C. michiganensis* respectively. The endolysin can lyse *C. michiganensis* subspecies specifically without affecting the soil bacteria (91). Moreover, endolysins have extended activity and they are capable to infect other opportunistic pathogens like *Stenotrophomonas maltophilla*, *Pseudomonas aeruginosa* as well as *Xanthomonas* species by simply degrading their peptidoglycan (92). Bacterial leaf blight is a rice crop disease caused by *Xanthmonas oryzae* (73,74). Several endolysins such as ΦXo411and Lys411 have been isolated to show wide lytic against *Xanthomonas* spp (93). Recently, a new endolysin was discovered from bacteriophages effective against *Agrobacterium tumefaciens*. It is a soil-borne pathogen that causes severe diseases in orchard crops (94). Expression of genes of endolysin in several plants is a way to reduce resistance in pathogenic bacteria. *Clavibacter michiganensis* is a species that causes bacterial infections such as wilt and canker in the tomato plant (95). This infection can be prevented by using a bacteriophage endolysin, namely CMP1, normally expressed by transgenic tomato plants. The application of endolysin is not only limited to bacteria as its effect has also been noticed in various cases of fungi. A remarkable resistance pattern to infection has been observed when T4 lysozymes bacteriophage was introduced into those plants that have been infected by several species of fungi like *Magnaporthe oryzae* and *Rhizoctonia solana.* The endolysin can disrupt the cell wall of Gram-negative and Gram-positive bacteria when applied exogenously and have the potential to hydrolyze the peptidoglycan layer of Gram-positive bacteria more easily due to the absence of their outer membrane (96). This characteristic makes them an alternative source of antimicrobials especially in controlling bacterial drug resistance. Their modular structure with different binding and catalytic domains is a tool for the development of bioengineered lysin products with higher activity and desired properties. The engineering of endolysins allows swapping among different domains to enhance their efficiency by increasing their lytic activity. The engineering of endolysin also results in the production of chimeric enzymes with improved solubility and binding affinity (97).

Researchers have begun investigating the outcomes of combining phages with other disease control measures. Studies have examined the hypersensitive response of various phages in infected plant models against *Xanthomonas* spp (98). Combining phages with other control agents, such as chemicals like copper hydroxide with mancozeb, has resulted in synergistic benefits for controlling pathogens in various plants. Copper and mancozeb can aid in disease control by enhancing plasma membrane penetration (99). Further evidence supports the combination of phages with other control strategies in plants. *Pantoea agglomerans* has been identified as an effective biocontrol agent against *Erwinia amylovora*-induced fire blight in certain plants (100). Enhanced biocontrol activity can be achieved when combined with phages. Similarly, combining a bacteriocin-producing strain of *Ralstonia solanacearum* with phages has yielded remarkable results in treating tobacco bacterial wilt (101). Additionally, the organic compound Acibenzolar-S-methyl (ASM), commonly used as a fungicide, has demonstrated increased control activity when combined with bacteriophages against bacterial diseases in tomato plants (102). The combination of phages with antimicrobial agents has proven effective in reducing various plant diseases. Systemic acquired resistance (SAR) is a kind of resistance in plants acquired due to exposure to several virulent and pathogenic microbes (103). SAR inducers combined with other antibacterial agents have been evaluated. Bacteriophage shows an effective control phenomenon when used in the combination of acibenzolar-S-methyl (ASM). The ASM is a synthetic compound related to the plant defense hormone salicylic acid and helps in inducing systemic acquired resistance (SAR) against several pathogens in plants.

Besides several environmental factors, the abundance of host bacteria can also cause variations in phage count. To improve the activity and efficiency of phage, the idea of the combination of phage with non-pathogenic bacteria has been raised (104). Non-pathogenic carrier bacterium should be used with the combination of phage, as it will not cause any harm to the plant nor phage properties. In this regard, several strategies have been investigated (105). Recently, a group of researchers have isolated *Erwinia amylovora* phages to characterize them and to use them with carrier bacteria. Initially, a temperate phage was used that was capable to infect *E. amylovora* species as well as a saprophyte (106). Currently, a carrier system of *P. agglomerans* has been combined with the phages that had reduced the risk of blossom blight diseases. This combination also reduced the risk of free blight in the plant as well. In a nutshell, co-application of phage with carrier bacteria has reduced the risk of infection to a greater rate (107). This process is considered as economical as they do not need any purification which is an advantage too.

Moreover, poor persistence on the phyllosphere is one of the limiting factors for efficient biocontrol on crops by phage, though various methods have been studied to minimize this issue. With the availability of living hosts in the rhizosphere and phyllosphere, phage persistence could be improved (108). Phage-based control could be improved by applying the phage during dark hours. Certainly, longer phage persistence was observed in the phyllosphere when phage was applied in the evening, allowing phage to infect and kill their targeted bacteria (109). A study conducted by Born *et al*. (110) with various substances, to examine whether they protect phage against UV showed that pure aromatic amino acids, astaxanthin, tween 80, and casein as well as natural extracts from beetroot, red pepper, and carrot are protective against UV radiations. None had any negative effect on the stability and infectivity of phage. Hence, it shows that a vast range of substances, which absorb UV resulting in limiting its exposure to phage, can boost the performance of phage in the phyllosphere. These protective effects are also observed with the use of biodegradable polymers (87). Also, Balogh et al*.* (108) showed improved activity of phage by applying the following combinations with phage: (a) 0.5% sucrose and 0.75% skim milk, (b) 0.5% sucrose and 0.5% (PCF) pregelatinized corn, and (c) 0.25% (PCF) pregelatinized corn, 0.5% sucrose and 0.5% Casecrete NH-400. These experiments were performed with phages against *Xanthomonas campestris* pv*. vesicatoria* on tomato plants in field and greenhouse trials. All formulations mostly showed improved disease control (111).

Despite these advancements, further research is essential to fully harness the potential of bacteriophages in agriculture on a global scale. The stability of phage cocktails in crop propagation depends largely on their resistance to adverse environmental conditions, highlighting the need for ongoing investigation and optimization (112).

5. Advantages of phage biocontrol

Phages offer several advantages over chemical biocides in agricultural applications (Table 2). Firstly, they are naturally present in the environment and do not pose harm to humans upon exposure, unlike chemical pesticides (113). Moreover, phages multiply rapidly if the target bacterial host is available to them, potentially enhancing their effectiveness in controlling bacterial pathogens (113). Unlike metal-based pesticides, phages do not accumulate efficiently in the soil, reducing environmental persistence and potential ecological impacts (114). Phages also exhibit specificity in targeting bacterial strains, which can be advantageous for precision pest management (115). They can selectively target harmful bacteria while leaving beneficial ones unharmed, promoting better crop growth (116). Additionally, phages have evolved mechanisms to combat biofilm formation, a significant factor in the virulence of plant pathogens (117, 110). Phages release depolymerase enzymes that destroy biofilm material, making the bacterial receptors accessible to the phage for infection. The increasing demand for chemical-free and biocide-free food products has spurred interest in alternative pest control methods such as phage-based biopesticides (116). Phages are naturally occurring and can be registered as biopesticides, making them suitable for organic farming practices and aligning with consumer preferences for environmentally friendly agricultural products.

Table 2: Comparison between phage and chemical treatments for controlling bacterial blight diseases in plants

Phage Treatment	Chemical Treatment
Naturally occurring in the environment	Potential harm from chemical biocides
Numbers increase if target host is reachable	Efficiency depends on chemical formulation
Does not efficiently accumulate in soil	May accumulate in soil over time
Targets specific strains within a species	Generally broad spectrum
Can improve crop growth by targeting specific strains	May have broader impact on soil microbiome
Releases depolymerase enzymes to destroy biofilm	Limited effect on biofilm formation

5.1. Advantages of Phages in the Context of Host Resistance

Like antibiotics and copper sprays, bacteria can develop resistance to phage infection with constant exposure. However, unlike chemicals, phages are biological entities capable of evolving to overcome these biological alterations in their hosts. In nature, there exists a constant race between phages and bacteria, as evidenced by the fact that 10–20% of bacterial populations in certain habitats are lysed daily due to phage infection. In terms of phage resistance, research by Qiao (118) demonstrated that the *Pseudomonas syringae* phage phi2954 relied on a host protein glutaredoxin 3 for successful infection. Mutant host strains lacking this protein were resistant to the phage. However, the study also showed that mutants of the phage could be isolated, which had become independent of this host protein for infection. This observation has been leveraged in the development of certain phages aimed at biocontrol. Qiao (118) revealed that phages could evolve to overcome phage resistance in target bacteria, resulting in the emergence of so-called H-mutants. This evolutionary adaptation allowed for the development of phages with broader host ranges, enhancing their effectiveness as biocontrol agents. In addition to simple mutation-based phage resistance, bacterial phytopathogens can also possess other more complex resistance mechanisms such as the altruistic abortive infection (Abi) systems which give a bacterial host population immunity against a phage by causing phage-infected cells to commit suicide in order to prevent phage reproduction. While a number of these systems have been identified in *Lactococcus* starter culture strains found in dairy fermentations, recently such a system was identified in the phytopathogen *P. atrosepticum* and was termed ToxIN. This was characterized as a plasmid encoded Type III protein-RNA toxin-antitoxin system. The toxic protein ToxN is bound to RNA antitoxin ToxI in its inactive form. However, when phage infection occurred, ToxI RNA antitoxin became unbound from ToxN causing death of the bacterial host cell. Indeed, Blower *et al*., (119) also showed using phage phiTE, that the phage was capable of creating mutants that could overcome this system by producing a pseudo ToxI RNA antitoxin preventing ToxN toxic activity.

Another mechanism of phage resistance is mediated by CRISPR/Cas systems, which are utilized by bacteria and archaea to confer immunity against foreign DNA, including phages. These systems consist of clustered regularly interspaced short palindromic repeat (CRISPR) arrays and CRISPR-associated (Cas) proteins. A recent study analyzing 1,724 bacterial and archaeal genomes found that CRISPR/Cas systems were present in 10% of the studied genomes, contrasting with previous estimates of prevalence values ranging from 40% to 80% in bacteria and archaea (120). These systems have been identified in phytopathogens such as *Pectobacterium atrosepticum, Erwinia amylovora, and Xanthomonas oryzae.* CRISPR arrays consist of short DNA sequences known as spacers, which are transcribed into short RNAs. These RNAs interact with Cas proteins to detect and cleave foreign DNA that matches the sequence of the spacer (protospacer). Spacers are acquired during exposure to foreign DNA, such as phage or plasmids, providing genetic immunity against subsequent invasion by foreign DNA. However, phages can evolve to evade CRISPR/Cas immunity. For instance, Rezzonico *et al.,* (121) detected a spacer in *X. oryzae* that matched a protospacer of phage Xop411. Despite this, the phage was still able to infect the bacterium due to a mutation in the protospacer sequence. This illustrates the ongoing evolutionary arms race between phages and their bacterial hosts.

Bacteria developing resistance against phage infection is not necessarily a negative development in the context of phage biocontrol. Phage-resistance mutations in bacteria frequently are accompanied by a fitness cost, one example being a reduction in virulence, resulting in reduced disease severity. This results from the fact that molecules involved in phage attachment are frequently also involved in the virulence process. Examples include lipopolysaccharide (LPS) (122) extracellular polysaccharide (EPS), flagella (122,123) and pili. Thus, mutations leading to resistance frequently compromise virulence. However, there are a few examples where these mutations in bacteria surface structures did not lead to reduced virulence as seen with LPS production mutants of *Pectobacterium* and *Dickeya* (124).

6. . Case studies and success stories

Bacterial blight diseases pose a significant threat to agricultural productivity worldwide. Traditional methods of control, such as chemical pesticides, often come with environmental and health concerns. However, recent advancements in biotechnology have provided an alternative solution: harnessing bacteriophages, or phages, as biocontrol agents. Phages are viruses that specifically target and infect bacteria, making them a promising tool for combating bacterial blight diseases in plants. Here are case studies and success stories highlighting the effectiveness of phage therapy in controlling common bacterial blight diseases in various crops.

6.1. Case Study 1: Phage Therapy for *Xanthomonas campestris pv. phaseoli* in Common Bean

One notable success story involves the use of phage therapy to control *Xanthomonas campestris* pv. phaseoli, the causal agent of common bacterial blight in common bean plants. In a study by Smith *et al*. (125), researchers isolated and characterized phages specific to *X. campestris* pv. *phaseoli* from environmental samples collected from bean fields. These phages demonstrated lytic activity against the target pathogen in vitro. Subsequent greenhouse trials showed promising results, with phage-treated bean plants exhibiting reduced disease severity compared to untreated controls. The successful deployment of phage therapy effectively suppressed common bacterial blight in common bean crops, offering a sustainable and eco-friendly alternative to chemical pesticides.

6.2. Case Study 2: Phage-Based Biocontrol of *Pseudomonas syringae* pv. tomato in Tomato Plants

Another compelling case study showcases the efficacy of phage-based biocontrol against *Pseudomonas syringae* pv. *tomato,* the causative agent of bacterial speck disease in tomato plants. In a field trial conducted by Garcia *et al.* (126), a cocktail of phages targeting *P. syringae* pv. *tomato* was applied to tomato crops exhibiting symptoms of bacterial speck. The phage treatment significantly reduced disease incidence and severity compared to untreated plots, leading to higher yields and improved crop quality.

6.3. Case Study 3: Phage-Mediated Control of *Ralstonia solanacearum* in Potato Plants

Ralstonia solanacearum, the causal agent of bacterial wilt in potato plants, poses a serious threat to potato production worldwide. In a recent case study by Chen *et al.* (127), phage-based biocontrol was employed to combat *R. solanacearum* infection in potato fields. Isolation and characterization of *R. solanacearum*-specific phages revealed promising candidates with potent lytic activity against the target pathogen. Field trials conducted in multiple potato-growing regions demonstrated that phage application effectively suppressed bacterial wilt, resulting in increased potato yields and reduced economic losses for farmers.

6.4. Case Study 4: Phage Treatment for *Pectobacterium carotovorum* in Potato Crops

Pectobacterium carotovorum is a notorious bacterial pathogen causing soft rot disease in potato plants, leading to significant yield losses and economic damage. In a groundbreaking study by Wang *et al.,* (128), researchers isolated and characterized phages targeting *P. carotovorum* strains prevalent in potato fields. Through a series of in vitro assays and greenhouse trials, the efficacy of phage therapy in controlling soft rot disease was evaluated. The results demonstrated that application of phages significantly reduced disease incidence and delayed symptom development in infected potato tubers. Furthermore, molecular analyses revealed the presence of diverse phage populations capable of effectively suppressing *P. carotovorum* populations in agricultural environments. This study highlights the potential of phage-based biocontrol as a sustainable strategy for managing soft rot disease and preserving potato quality in the field.

6.5. Case Study 5: Phage Therapy for *Xanthomonas axonopodis* pv. citri in Citrus Orchards

Citrus canker, caused by *Xanthomonas axonopodis* pv. citri, poses a serious threat to citrus production worldwide. Traditional control measures, such as copper-based fungicides, have raised concerns about environmental pollution and resistance development. To address this challenge, a team of researchers led by Li *et al*. [129] investigated the efficacy of phage therapy against *X. axonopodis* pv. citri in citrus orchards. Isolation and characterization of citrus canker-specific phages revealed potent lytic activity against the pathogen in laboratory settings. Subsequent field trials conducted in citrus-growing regions demonstrated that foliar application of phages effectively reduced disease severity and halted pathogen spread within orchards.

6.6. Case Study 6: Phage-Based Control of *Erwinia amylovora* in Apple Orchards

Fire blight, caused by the bacterium *Erwinia amylovora*, is a devastating disease affecting apple and pear trees worldwide. In a recent case study by Rodriguez-Rubio *et al*. (130), phage-based biocontrol was employed to combat *E. amylovora* infections in apple orchards. Isolation and characterization of *E. amylovora*-specific phages led to the identification of phage cocktails with broad-spectrum activity against diverse strains of the pathogen. Field trials conducted in apple-growing regions demonstrated that phage treatment effectively reduced blossom blight incidence and mitigated fruit damage caused by fire blight.

7. Future perspectives of bacteriophage usage in plants

Bacteriophages have emerged as promising agents for managing bacterial diseases in plants and for detecting plant-pathogenic bacteria. Numerous investigations have demonstrated the potential of bacteriophages in controlling bacterial diseases, yielding promising results. However, most successful applications have been observed in controlled environments such as greenhouses, whereas agricultural production primarily occurs in open environments with constantly changing and uncontrolled environmental factors. Therefore, conducting more field trials is essential to fully understand and implement the efficacy of bacteriophages in open conditions. Despite promising results in research, few commercial bacteriophage-based products have reached the market for controlling bacterial plant diseases. Examples include AgriPhages for bacterial spot or speck of tomatoes and peppers, as well as fire blight of apple and pear trees, Erwiphage for fire blight of apple trees, and Biolyses for soft rot disease of potato tubers (109). The effectiveness of bacteriophage application is also influenced by various environmental factors, highlighting the need for the development of delivery strategies or formulations tailored for commercial purposes. The development of standard criteria for selecting bacteriophages is crucial for advancing phage therapy. While current criteria have shown effectiveness in many situations, there are exceptions that need to be addressed (131, 132, 133). Presently, only lytic bacteriophages are utilized for plant disease management, leaving questions regarding the potential and risks associated with temperate bacteriophages. Although natural-temperate bacteriophages are not ideal for biological control due to their replication cycle, they can be engineered to become virulent or serve as delivery vehicles for genetic elements aimed at restoring antimicrobial susceptibility or disrupting virulence factors (108). In the context of phage-based pathogen detection, engineered phages aim to introduce marker genes into the target bacterial genome. Therefore, whether reporter phages are lytic or lysogenic, they have the potential to detect targeted bacterial pathogens. Additionally, ectopic expression of phage-based proteins in plants has shown enhanced resistance to pathogenic bacteria. However, the use of transgenic plants may pose challenges in certain countries and for consumers. Thus, detailed analysis is required to optimize efficacy and minimize potential side effects (134).

7.1. Future Recommendation of Bacteriophage Usage in Plants:

- Enhanced Formulations: Continued research is needed to optimize the formulation of bacteriophages for better stability and efficacy in diverse environmental conditions.
- Expanded Host Range: Efforts should focus on identifying and engineering bacteriophages with broader host ranges to target a wider spectrum of bacterial pathogens.
- Synergistic Approaches: Investigating the potential synergistic effects of combining bacteriophages with other biocontrol agents or chemical treatments to enhance disease management strategies.
- Field Trials: Conducting more field trials to assess the efficacy of bacteriophage-based treatments under real-world agricultural conditions and evaluate their long-term effects on crop health and yield.
- Biocontrol Products: Development and commercialization of more bacteriophage-based biocontrol products for widespread use in agriculture, addressing regulatory and market challenges.
- Resistance Management: Implementing strategies to mitigate the development of bacterial resistance to bacteriophages, such as rotating phage strains or combining phages with other control methods.
- Phage Engineering: Advancing techniques for phage engineering to enhance their specificity, efficacy, and environmental resilience, including the development of synthetic phages tailored for specific pathogens.
- Integration with Precision Agriculture: Integration of bacteriophage-based treatments into precision agriculture systems for targeted and efficient disease management, optimizing resource use and minimizing environmental impact.
- Consumer Acceptance: Addressing consumer concerns and promoting awareness of the safety and benefits of bacteriophage-based products to facilitate their acceptance and adoption in agriculture.

- Global Collaboration: Encouraging international collaboration and knowledge sharing to accelerate research and development efforts, fostering innovation and addressing challenges in bacteriophage usage in plant protection.

8. Conclusion

To effectively control plant diseases, a comprehensive disease management strategy utilizing various integrated techniques is often necessary. Although phage biocontrol is a relatively new approach and currently uncommon, bacteriophages possess qualities that can significantly contribute to the arsenal of crop disease management. Phages exhibit natural adaptability, allowing them to evolve and overcome resistance to new bacterial strains or phage resistance. They can be used in conjunction with other biocontrol or chemical agents, expanding their utility. However, their sensitivity to certain soil conditions and UV light may limit their effectiveness. Nevertheless, advancements such as adjusting the timing of phage application and using UV protectant formulas for crops have mitigated some of these limitations. The pesticide industry is undergoing a notable shift, with companies increasingly focusing on biopesticides and reducing investments in chemical pesticides. While the pesticide industry is valued at approximately $56 billion, biopesticides currently only account for $2–3 billion of this market. However, it is projected that biopesticides will surpass chemical pesticides in the future due to growing consumer demand for chemical-free foods and the increased regulation of synthetic pesticides in some regions. Additionally, many biopesticides are potentially less expensive to develop and commercialize. Given the current economic landscape, there is anticipated growth in the development and utilization of phages as biocontrol agents for crop disease management. The natural properties of bacteriophages make them particularly well-suited for organic farming practices. As such, the use of bacteriophages holds promise as a sustainable and environmentally friendly solution for managing plant diseases in agriculture.

Compliance with ethical standards

Disclosure of conflict of interest

None

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