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REVIEW ARTICLE

Harnessing the bacteriophages to deal with canker infection of kiwi fruit: challenges and future perspective

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ABSTRACT

The worldwide kiwifruit industry has suffered significant economic losses in recent decades as a result of the destructive bacterial plant disease *Pseudomonas syringae* pv. *actinidiae* (Psa). Existing control approaches, which depend on the use of copper bactericides and antibiotics, are facing growing challenges due to the rise of resistance to antibiotics and ecological issues. Although biocontrol techniques show promise in laboratory settings, their efficacy in real-world field situations remains unclear. In order to tackle this issue, the emergence of a phage-based biocontrol method becomes a vital alternative, considering the precise targeting of bacteriophages (phages) towards the particular bacteria and their ecologically benign characteristics. This thorough assessment commences by delineating the repercussions of Psa-induced kiwifruit canker, underscoring the need for pioneering management strategies. The text explores the many types and strengths of Psa strains, and then shifts its attention to recent progress in the identification and description of Psa phages. The main topics discussed are the physical structure of phages, the spectrum of organisms they may infect, their ability to destroy bacterial cells, the study of their genetic material, and the process by which they break down bacterial cells. The review examines biocontrol tactics and their possible obstacles in kiwifruit orchards, specifically focusing on abiotic variables such as elevated temperature, UV irradiation and severe pH. The manuscript highlights the crucial importance of phages in efficiently controlling Psa infections, providing a sustainable and focused approach for safeguarding plants.

KEYWORDS

Bacteriophages, *Pseudomonas syringae* pv. *actinidiae* (Psa), lytic activity, phage therapy, environmental adaptation

INTRODUCTION

Lorem Kiwifruit is widely recognized because of its distinctive taste and notable nutritional attributes, notably its significant vitamin C concentration (Chen *et al*., 2020). The worldwide kiwifruit production is at approximately 1.5 million tons per annum. China, Iran, Italy, Chile and New Zealand are the primary nations engaged in kiwifruit production, collectively accounting for over 90% of the global kiwifruit output (Sharma, Kumar, & Kumar, 2022). The canker, triggered by *P. syringae* pv. *actinidiae* (Psa), has been well recognized as the most destructive pathogen affecting kiwifruit production. Several kiwifruit-growing countries have reported significant losses attributed to this illness (Kim & Koh, 2019; Savian, 2020). PSA infection has been found to induce a range of symptoms and, in more severe instances, can lead to the demise of plants, resulting in a substantial decrease in both the quantity and quality of kiwifruit production (Donati *et al*., 2020).

The isolation of prostate-specific antigen (PSA) was initially documented in Japan in the year 1984. The global dissemination of this information encompassed various nations, including China, Korea, Georgia, Italy, Spain, Portugal, Turkey, Greece, France, Slovenia, New Zealand, Chile, Australia and Switzerland (Patra & Roy, 2023). The rapid dissemination of the virus responsible for the disease epidemic can be largely ascribed to the clonal proliferation of kiwifruit, facilitating its transmission through seedlings (Panno *et al*., 2021). Significantly, the prevalence of this phenomenon might be attributed to various factors, such as the extensive phenotypic and genetic variability observed across the Psa isolates, alongside the introduction of novel virulent strains. Isolates of Psa bacteria have been categorized into six distinct biovars, namely biovars 1, biovar 2, biovar 3,biovar 4, biovar 5 and biovar 6, through the assessment of virulence and biochemical traits (Lee, Kim, Koh, & Jung, 2020; Tahir *et al*., 2019). Among the several strains, those belonging to biovar 4 were transferred to the newly identified pathovar actinidifoliorum because of their comparatively lower level of aggressiveness. This characteristic sets them apart from the other biovars of *P. syringae* pv. *actinidiae* (Psa). Moreover, it has been observed that Psa strains classified under biovar 3 play a significant role in the worldwide outbreak of kiwifruit cankers.

1. Significance of canker infection in kiwi fruit cultivation

P. syringae pv. actinidiae (Psa) is the pathogen that causes canker infection, which poses a threat to kiwi fruits and has an impact on both the agronomy and the economics of this sector (Luo *et al*., 2022). This bacterial disease was discovered for the first time in Japan in the 1980s. Since then, it has spread all over the world, causing significant damage to kiwi orchards in key producing nations such as New Zealand, Italy, China, and Japan (Mazzaglia *et al*., 2012). The infection can cause leaf spots, cane dieback, and oozing cankers, all of which can lead to significant output losses and, in extreme situations, the death of the kiwi vines. An illness caused by a canker has a severe influence on the economy. The use of bactericides, the cutting of sick material, and even the removal of whole orchards are all expensive methods of disease management, and farmers sometimes struggle to afford the expenditures associated with these methods. In addition to this, the loss of yields that occurs as a result of this illness directly reduces the money that is generated from the sale of fruits; hence, this becomes a significant problem for farmers that rely heavily on kiwi farming as their primary way of creating cash. Another example of its influence on the economy is the amount of money that is spent on illness management. During the first few years after the outbreak, the expenditures of maintaining and doing research to control Psa in New Zealand reached over fifty million New Zealand dollars. The research on resistant kiwifruit types that was carried out as part of this spending led to the creation of new cultivars such as 'SunGold' that demonstrated better levels of resistance to Psa (Dwiartama, 2017).

The public service announcement (PSA) has been identified as a significant factor contributing to substantial global economic losses, a trend that

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persists to this day. The land worth of cultivating orchards widely consumed kiwifruit cultivar Hort-16A that experienced a significant decline, going from 300,000 to 46,000 USD per hectare. This has had a substantial negative impact on the economy of New Zealand. According to the data obtained from the source (World's Top Exports, accessible on 1 November 2022), it can be observed that New Zealand emerged as the leading exporter of kiwifruit in 2021. The entire export value of kiwifruit from New Zealand amounted to USD 2 billion, which accounted for around 50.9% of the overall kiwifruit exports (Camiring, 2022). In the context of Psa infection, it has been observed that effective mitigation can be achieved through the thorough description of the pathogen and the subsequent creation of a highly sophisticated detection approach. In addition, the utilization of copper bactericide and streptomycin has been extensively employed as a means to mitigate the detrimental effects caused by this particular ailment. However, their effectiveness in managing Psa infection was found to be less reliable when tested in real-world conditions. It is noteworthy that there has been a notable increase in the inclination towards utilizing phage therapy as a means to regulate plant bacterial infections. Several phage products, including Agri-Phage-TM and Eco-Shield-TM, have been successfully developed and introduced into the commercial market (Wójcicki, Błażejak, Gientka, & Brzezicka, 2019).

This article provides a comprehensive overview of the recent advancements in utilizing bacteriophages (phages) for the control of bacterial canker ailment in kiwifruit. Additionally, it discusses the potential obstacles associated with phage therapy and explores its prospects.

2. Psa Phages Characterization

2.1. Isolation of Psa phages

Bacteriophages belonging to the Psa genus have been effectively obtained from various ecological habitats across the globe. In a study conducted by Yin *et al*. (2019), a total of 36 Psa phages were identified and purified from the kiwifruit orchards located in the primary production region of China (Yin *et al*., 2019). Furthermore, it has been observed that the isolated phages exhibit a diverse range of morphological characteristics. For instance, Huang *et al*. (2013) stated that form all isolated Psa phages 6.3% had siphovirus, 41.7% had podovirus, and 52.1% had myovirus morphology (Huang *et al*., 2013). In addition, Liu *et al*. successfully obtained a novel lytic phage, PHB09, from the soil of a kiwifruit orchard located in Sichuan, China (Liu *et al*., 2021). This phage exhibits distinct traits that differentiate it from the established myovirus groups, suggesting that it should be classified under a newly defined genus. Recent investigations have demonstrated that phages, albeit exhibiting diverse host ranges, exhibit significant promise in the management of kiwifruit bacterial canker pathogens within orchard settings (Buttimer *et al*., 2017).

2.2. Morphological characterization

Based on morphological examination conducted by transmission electron microscopy (TEM), it has been determined that the phages belonging to the Caudoviricetes class make up the predominant portion, exceeding 97%, of *Pseudomonas* phages. Based on the characterization of tail form, it is observed that the phages from Caudoviricetes generally exhibit three unique morphological characteristics. Phages possessing the podovirus type have small tails, whilst the myovirus phages are distinguished by their tails which are double-layered and contractile. On the other hand, the siphovirus phages have flexible and elongated tails. Furthermore, the examination of the phages' heads through morphological observation indicated that they can be categorized into two distinct shapes across all families, either icosahedral or oblate (Yang *et al*., 2022). The data in Table 1 provides details about Psa phages. For instance, the classification of both PN09 and PHB09 as myoviruses was determined by analyzing their morphological properties, which were acquired through the use of transmission electron microscopy (TEM).

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Table 1. Morphological characteristics of some Psa phages.

Name	Class	Head Size (µm)	References
Φxwy-0013	Siphovirus	0.073	(Yin *et al*., 2019)
Φxwy-0014	Myovirus	0.070	(Yin *et al*., 2019)
Φxwy-0026	Podovirus	0.080	(Yin *et al*., 2019)
ΦPsa-17	T7-like Podovirus	0.056	(Frampton *et al*., 2014)
ΦPsa-173	Siphovirus	0.078	(Frampton *et al*., 2014)
KHUΦ-34	Myovirus	0.090	(Yu *et al*., 2016)
PHB-09	New Myovirus	0.055	(Liu *et al*., 2021)
phiPSA1	Siphovirus	0.060	(Di Lallo *et al*., 2014)
PsageK9,B1,B2	Siphovirus	0.078	(Martino *et al*., 2021)
PsageK4	Myovirus	0.072	(Martino *et al*., 2021)
Psage A2	Myovirus	0.072	(Martino *et al*., 2021)
Psage A1	Myovirus	0.072	(Martino *et al*., 2021)
Psage K4e	Myovirus	0.072	(Martino *et al*., 2021)
KHUΦ59	Podovirus	0.069	(Yu *et al*., 2016)

2.3. Range of hosts

The host range of a bacteriophage refers to the systematic breadth of hosts that it is capable of effectively infecting. Comprehending the breadth of the host range is of utmost significance to optimize the efficacy of Psa phages (Luo *et al*., 2022). Numerous studies have provided evidence suggesting that the majority of Psa phages exhibit a limited range of hosts when it comes to infecting the Psa pathogen (Zeaki, Johler, Skandamis, & Schelin, 2019). In general, the limited host specificity of bacteriophages results in the infection of only harmful bacterial strains. As demonstrated by Ni *et al*. (2020), the phage PN-09 could effectively kill all 29 strains of Psa (Ni *et al*., 2020). However, it did not demonstrate the same lytic activity towards other bacteria that were distantly related. In a similar vein, the outcomes of the host range experiments demonstrated that phage PHB-09 could cause lysis in biovar 2-3 strains of Psa, while failing to infect the remaining *Pseudomonas* sp. strains that were subjected to testing. In contrast, Flores *et al*. (2020) demonstrated a variation in the host range of the phages associated with Chilean Psa. As an example, it was shown that phage CHF-33 demonstrated a higher level of lytic action against various Psa isolates compared to phage CHF-1. Nevertheless, it was found that all of the tried isolates of Psa might be effectively lysed by employing a combination of the selected phages (Flores *et al*., 2020).

The host range of bacteriophages is contingent upon the specific phage and the bacterial host. For instance, the isolates of Psa obtained from New Zealand, Japan, Korea and Italy displayed varying degrees of susceptibility to phages with different titers. The findings of Di Lallo *et al*. (2014) demonstrated that the fPSA-1 exhibited the capability to induce lysis in Psa isolates exclusively, while its ability to induce lysis in other pseudomonads was absent (Di Lallo *et al*., 2014). These results imply that the host range of the Psa phage fPSA1 is highly limited. On the contrary,

several bacteriophages have been documented to possess the capability to infect diverse combinations of *P. syringae* pv. *actinidiae* (Psa) isolates originating from distinct geographic regions. As an example, Frampton *et al*. (2015) documented that phage φPsa17 could taint a diverse array of Psa strains, encompassing those obtained from Italy, Japan, South Korea, New Zealand (Frampton *et al*., 2015).

2.4. Lytic activity

The lytic activity of bacteriophages is frequently assessed by the utilization of a one-step growth curve, which encompasses various key parameters like the latent time, the rising duration, and burst size (Liao *et al*., 2019). The study found a positive correlation between lytic activity and burst size, while a negative correlation was observed between lytic activity and both the latent phase and the rising phase. The magnitude of the burst size holds significant importance as it directly correlates with the quantity of viral particles generated, hence indicating the possibility for further cellular infections (Kannoly *et al*., 2023). After conducting the single-step experiment, it was observed that the lytic Psa phages had a growth curve with an S-shape, which was distinguished by a substantial burst size and a brief latency period. This suggests that the replication of all phages was successful within the bacterial cells they infected. According to the data presented in Table 2, notable variations were seen in the latent time, rising period, and burst size across the Psa phages. As an illustration, it was observed that the lysogenic fPSA1 shown a prolonged time of latency and rise, accompanied by a substantial burst size. Conversely, the lytic fPSA2 demonstrated a brief latency and rise, accompanied by a smaller burst size. In order to effectively address plant ailments through the utilization of phages, either as standalone agents or in combination, it is imperative that the phages possess lytic activity. This prerequisite is essential for the successful implementation of phage treatment. Therefore, the aforementioned data suggest that all phages capable of lysing Psa strains exhibit potential as viable candidates for the application of phage therapy in the treatment of kiwifruit cankers (Luo *et al*., 2022). In contrast to the aforementioned lysogenic phages, the majority of lytic phages demonstrate a high efficacy in bactericidal activity.

3. Infection mechanism

The process of lytic phage infection in host bacteria encompasses several steps, including phage attachment to host cells, DNA injection into host followed by self-replication within the host cells, ultimately resulting in the demise of the host cells (Luo *et al*., 2022). The endolysin and holin, imparts lytic ability to bacteriophages against host bacteria, which are recognized as two enzymes of bacteriophages. Endolysins and Holins have been extensively documented for their ability to cause damage to the peptidoglycan layer and the inner cell membrane, respectively. For example, there have been reports indicating that the phage endolysin (a phage PN09 derived enzyme) LysPN-09 exhibits an ability to induce cell lysis to host bacteria through efficient degradation of principal organizational constituent of the bacterial cell wall, the murein sacculus. In contrast, the role of endolysin is to puncture the inner cell membrane and establishing the precise timing for bacterial cell lysis.

In recent times, there has been a growing focus on the discovery of novel phage endolysins and their prospective applications in the field of agriculture as antibacterial agent (Lai, Chen, Ho, Xia, & Leung, 2020). In a study, the researchers investigated the efficacy of the endolysin LysPN-09 from phage PN-09 in combination with EDTA. The results showed that this combination successfully infected all 29 tested strains of *P. syringae* pv. *actinidiae* (Psa) and had robust action against Psa cells. Moreover, the treatment with LysPN09 and EDTA resulted in the permeabilization of the outer membrane of Psa cells, and the combination exhibited favorable stability under varying temperature and pH conditions. Alternatively, additional pathways for phage lysis have been suggested. As demonstrated by Ni *et al*. (2020), the biofilm development of Psa strains was successfully suppressed by the application of phage suspensions, whether consisting of a single phage or

a combination of phages (Ni *et al*., 2020). The primary factor responsible for the elimination of biofilms is the enzymatic activity exhibited by phages, which enables them to actively disrupt and decrease the production of bacterial biofilms in their host organisms (Kranjec *et al*., 2021). In addition, lytic bacteriophages have the ability to induce their host bacteria to increase the production of EPS-degrading enzymes, hence enhancing the ability of phages to infiltrate and traverse the bacterial biofilm of the host. Following this, bacteriophages initially enter host bacterial cells by means of the biofilm, subsequently replicate within the bacterial cells of their host, and ultimately eradicate host bacteria through lytic action (Chegini *et al*., 2020).

Table 2. The characteristic Psa phages exhibit lytic behaviour.

Phages	Mode of Action	Latency Period	Rise Duration	References
ϕXWY-0013	lytic	120 sec	210 sec	(Yin *et al*., 2019)
ϕXWY-0014	lytic	90 sec	210 sec	(Yin *et al*., 2019)
ϕXWY-0026	lytic	180 sec	300 sec	(Yin *et al*., 2019)
PN-09	lytic	120 sec	600 sec	(Ni *et al*., 2021)
fPSA-1	lysogenic	600 sec	300 sec	(Di Lallo *et al*., 2014)
fPSA-2	lytic	90 sec	90 sec	(Di Lallo *et al*., 2014)
Φ6	lytic	600 sec	120 sec	(Pinheiro *et al*., 2020; Pinheiro, Pereira, Frazão, Balcão, & Almeida, 2019)
PHB-09	lytic	360 sec	240 sec	(Liu *et al*., 2021)

4. Environmental stress tolerance

In order to achieve efficient management of bacterial canker illness, it is important to assess the phage activity within several ecological settings present in kiwifruit orchards. This assessment serves as a crucial determinant for the successful implementation of phage therapy as a control measure. Psa phages exhibit susceptibility to a range of environmental factors, including elevated temperatures, ultraviolet (UV) light exposures and unusual pH levels. Despite the potential of phages as a biocontrol agent for bacterial cankers, their effectiveness and practicality in field applications have been hindered by population decline caused by several circumstances (Grace, Rabiey, Friman, & Jackson, 2021). The postulated processes of these abiotic aspects have suggested that Psa phages experience inactivation due to structural damage to their components and/or the induction of alterations in DNA structure. Consequently, there is a reduction in phage titers within the phyllosphere. It is worth noting that the decrease in phage activity is contingent upon the particular strain of the phage, with certain strains exhibiting greater tolerance than others. Hence, it is imperative to prioritize the thorough examination of phages' environmental adaptation aspects when selecting them for the purpose of biocontrol against bacterial canker disease of kiwifruit in agricultural settings (Córdova *et al*., 2023).

Temperature and pH are recognized as significant environmental parameters that impact the viability and robustness of phages. These factors exert their influence by affecting many stages of the phage life

cycle within host bacterial cells. Certain phages exhibit the ability to introduce the genetic material into host cells exclusively under low temperature conditions. Conversely, elevated temperatures facilitate the degradation of capsid proteins, thereby leading to an extended time of phage latency (Zhang, Weinbauer, & Peduzzi, 2021). According to many reports, the lytic activity of most Psa is shown to be optimal within a pH range of 6 to 8. Nevertheless, it is worth noting that many Psa phages have been observed to exhibit tolerance towards a broad spectrum of pH levels, spanning from 2.0 to 12.0 (Luo *et al*., 2022).

The impact of UV irradiation on phage activity in the plant phyllosphere, leading to reduced lifetime, has been extensively acknowledged in the natural environment (Mandal *et al*., 2023). UV light has been shown to cause direct harm to free viruses through the degradation of proteins in phage particles, alteration of nucleic acid structure, and reduction of phage infectivity. The study revealed that shorter wavelengths have an irreversible impact on genetic material, leading to the alteration of phage proteins and the creation of fatal photo-products. DNA phages typically exhibit heightened susceptibility to UV radiation as a result of the generation of deleterious photoproducts generated by UV radiation such thiamine dimer. Conversely, dsRNA or dsDNA phages have enhanced resilience to these rays in comparison to ssRNA or ssDNA phages (Mandal *et al*., 2023). Nevertheless, the susceptibility of phage prion to UV radiation can be mitigated through the use of various strategies, such as employing high-concentration phages during periods of reduced radiation, such as in the morning or at night.

5. Phages as disease controlling agents

5.1. Lytic activity

Numerous Psa phages, which have demonstrated robust lytic activity against their bacterial hosts, have been effectively isolated in various nations including China, Italy, Japan, New Zealand and Korea (Martino *et al*., 2021). The Psa phages that were acquired demonstrated several advantageous attributes, including significant lytic action against the host cell (bacteria), minimal or no impact on other bacteria residing the soil microbiota, and resilience to diverse environmental pressures. Yin *et al*. (2019) shown that the 36 phages acquired in their study exhibit a notable level of selectivity towards Psa strains that are classified under biovar 3 (Yin *et al*., 2019). In addition, Yu *et al*. (2016) demonstrated the stability of 5 Psa phages under varying pH conditions and exposure to UV-B radiation (Yu *et al*., 2016). In their study, Ni *et al*. (2021) documented that phage PN09 exhibited the capability to effectively lyse all 29 strains of Psa belonging to biovar 3 (Ni *et al*., 2021).

5.2. Biocontrol potential

Various researches have elucidated the ability of Psa phages for the purpose of biologically controlling Psa disease in kiwifruit plants. As an example, Flores *et al*. (2020) conducted an isolation of four Podovirus phages, namely CHF1, CHF7, CHF19, and CHF21 (Flores *et al*., 2020). Their findings indicated, when these phages were employed individually or in combination, resulted in a noteworthy reduction in Psa symptoms inside a greenhouse environment. The findings from the study conducted by demonstrated that phage PHB09 founded to be environmentally stable and effectively mitigates bacterial canker infections in kiwifruit. These results suggest that phage PHB09 holds significant promise for application in the field of biological management against Psa infection. In addition, Pinheiro *et al*. (2019) observed that the bacteriophage phi6 exhibited a decrease in cellular density of various Psa strains under laboratory settings (Pinheiro *et al*., 2019). In their study, Flores *et al*. (2020) shown that the 4 phages that were identified exhibited significant promise for the use of biological management against Psa infection in greenhouse settings. In their study, Song *et al*. (2019) documented the successful utilization of the Podovirus phage PPPL-1, which was obtained from Korea, for the purpose of efficiently managing bacterial cankers in kiwifruit (Song *et al*., 2021).

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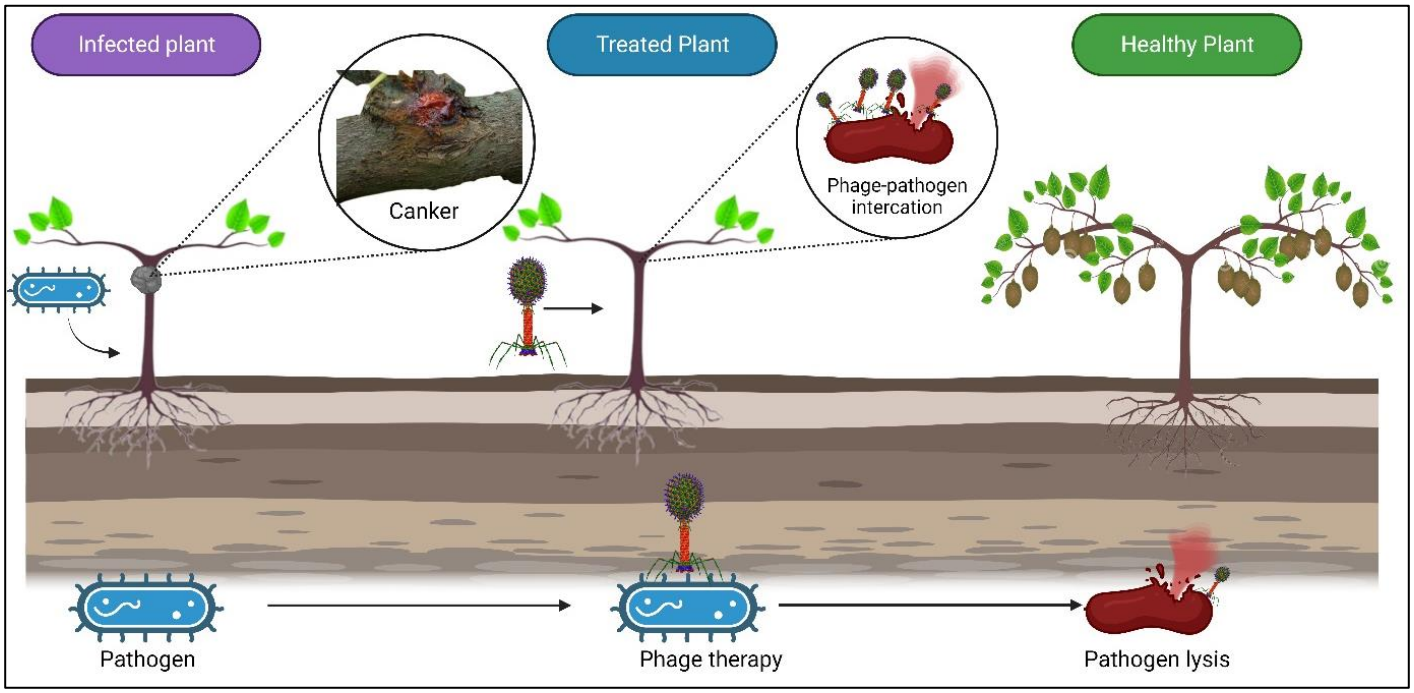

Figure 1. *A schematic view of phage therapy to control the Psa infection.*

5.3. Phage cocktail

The outcomes of the host range investigation demonstrated that Psa strains originating from various geographical locations were susceptible to lysis by the majority of isolated Psa phages. An illustration of this may be seen in the study conducted by Flores *et al*. (2020), where the authors demonstrated the high efficacy of six specific podovirus phages in lysing various Chilean *P. syringae* pv. *actinidiae* (Psa) isolates. Moreover, Frampton *et al*. (2015) reported that the phage φPsa17 had a reasonably wide host range, demonstrating the ability to lyse Psa strains originating from Japan, New Zealand, South Korea and Italy (Frampton *et al*., 2015). In addition, it was observed that the pathogenic variants of *P. syringae* and other phages of *Pseudomonas* had the ability to effectively lyse cells of certain strains of Psa. This finding implies that these phages hold promise as viable candidates for the development of phage cocktails to combat Psa infection in the future. The findings from the study conducted by Pinheiro *et al*. (2019) shown that the phage Φ6 exhibits the ability to infect not only its native host Psa (Pinheiro *et al*., 2019). Nevertheless, it is worth noting that in certain instances, the isolated bacteriophages exhibited a high degree of specificity towards particular strains of Psa that fall under biovar 3. This specificity significantly limits the potential applicability of these phages for the purpose of controlling bacterial canker in kiwifruit.

However, the rise of bacteria that are resistant to phages poses a significant obstacle in utilization of bacteriophages for the management of Psa diseases. Multiple researches have demonstrated that the utilization of phage mixtures or combination cures can enhance the efficacy of phage remedy and mitigate the development of resistance to phages. An example study conducted by Song *et al*. (2021) demonstrated the successful suppression of canker infections in kiwifruit caused by bacterial through the application of a combination of phage PPPL-1, KHUΦ34, and KHUΦ38 (Song *et al*., 2021) (also shown in Fig.2). The researchers found that pre-treating with the PPL-1 phage was equally effective in controlling bacterial cankers in kiwifruit as using antibiotic products.

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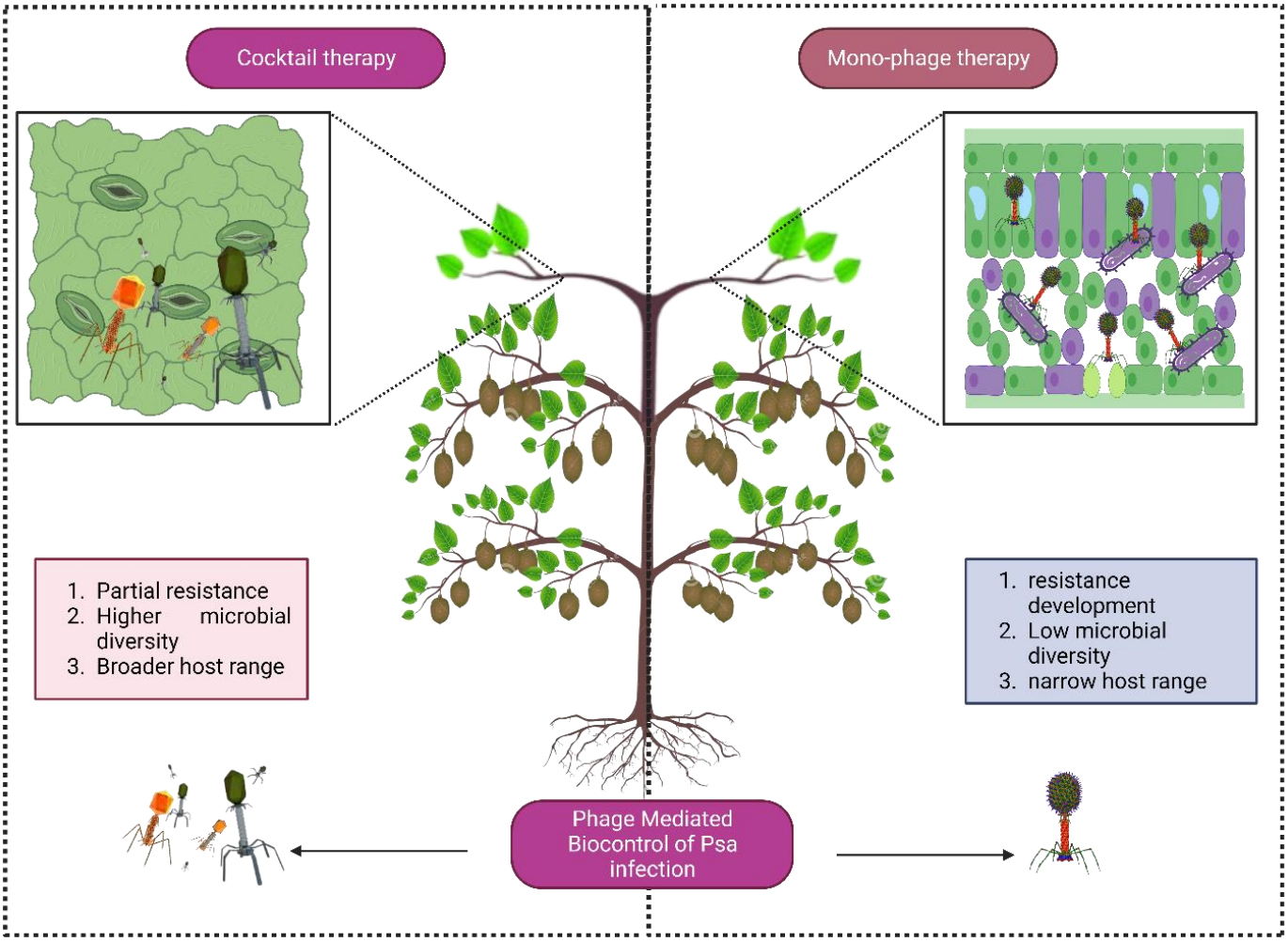

Figure 2. *Comparison between efficacy of mono-phage therapy and phage cocktail therapy.*

5.4. Advantages

Phages are regarded as highly capable, harmless, and effective substitutes for the prevention of cankers in kiwifruit plants, owing to their limited host range, in contrast to copper-based bactericidal (Di Lallo *et al*., 2014). Bacteriophages are widely recognized as one of the most prevalent forms of biological beings found on our planet, with an estimated number ranging from 10^{30} to 10^{31} inside the biosphere. The ease of isolating phages with potential applications in the control of plant pathogenic bacteria is attributed to their inherent and widespread occurrence in the biosphere. It is worth mentioning that a majority of phages targeting *P. syringae* pv. *actinidiae* (Psa) shown an inability to cause lysis in other bacterial species obtained from the normal habitat of kiwifruit plants. Nevertheless, other researchers have documented that Psa phages has the capability to induce lysis in additional strains of *P. syringae*, including different species of *Pseudomonas*. Phages are observed as safe agents for targeting specific pathogens, as they do not exhibit any detrimental impact on plant cells or other advantageous microbes (Kazi & Annapure, 2016). Conversely, it is possible to enhance the host range of phages by developing phage cocktails targeting different plant pathogenic bacteria within the *P. syringae* species complex, so potentially creating a synergistic effect.

6. Challenges

Phage therapy has been recognized as a viable approach for managing *Pseudomonas syringae* pv. *actinidiae* (Psa) infections in kiwifruit plants. Nevertheless, the utilization of Psa phages for plant protection continues to present various obstacles and constraints.

6.1. Specificity

The Psa phage has a notable degree of specificity, enabling it to exclusively infect its intended host bacteria (Hagens & Loessner, 2010). Conversely, many biovars of the Psa bacteria demonstrate variations in their sensitivity to phage infection. Nevertheless, certain instances have revealed a significant level of intraspecies variability among the specific Psa pathogens as a result of localized adaptation. It is noteworthy that the utilization of phage cocktails has the potential to significantly enhance the lytic capacity of host bacteria through the deliberate selection of phages with a limited host range (Żbikowska, Michalczuk, & Dolka, 2020). In addition, in order to facilitate the creation of tailored phage cocktails capable of targeting agriculturally significant plant pathogenic bacteria, it is imperative to establish an extensive repository of lytic phages. Furthermore, it is imperative to regularly isolate and gather novel phages in order to acquire a comprehensive collection of phage resources that exhibit high efficacy against recently emerged mutant and resistant strains.

6.2. Genetic Engineering

The sequencing of additional Psa phage genomes has contributed to the enhancement of phage biocontrol efficacy through the application of genetic engineering (Kilcher, Studer, Muessner, Klumpp, & Loessner, 2018). This approach has recently been suggested as a viable method to augment the action of lytic phages. There are many methods that may be used to get these new phages, such as homologous replication, physical mutation, CRISPR-Cas editing of genes, phage recombining of electroporated DNA. The changes in the receptor-binding molecules of readily accessible phage φ6 have mostly been associated with modifications in the spectrum of hosts it may infect. The contamination of phage φ6 in a similar species of *Pseudomonas* can be linked to the increased rate of mutation found in its genome, which allows it to gain new habitats. Furthermore, to their infection, the phages possess the potential for genetic engineering to enhance their resilience against many environmental stressors, hence augmenting their viability when deployed in orchards. The utilization of genetically edited phages presents a possible hazard to biological security due to the absence of regulatory measures governing the potential dissemination of these modified phages into the surrounding ecosystem (Piergentili *et al*., 2021).

7. Future perspectives & conclusion

The agricultural industry places a high priority on eradicating Psa in kiwifruit orchards; yet, there are currently few, ineffective, and ecologically unsafe authorised therapies for this disease. While streptomycin and copper formulations remain the gold standard for Psa control, a number of additional substances and procedures have shown promising results, offering potential alternatives or supplements to these approaches. There has been a significant uptick in the study of phages as a potential new, less harmful way to combat bacterial infections. Some phage-based solutions are already hitting the market, and their application offers a practical way to manage certain harmful bacterial crop diseases. Despite the fact that phages have been shown to effectively treat plant bacterial illnesses in several studies, the efficacy of phages in controlling Psa infections has received little attention. Phage inactivation of Psa has only been investigated in five research so far, all of which used either in vitro or ex vivo tests. Additional research with whole kiwifruit plants is required to use this method in the field; first, on a small scale in a controlled environment, and then, in naturally polluted orchards. To better understand the phage-host interactions, isolate different lytic phages for different pathogen biovars, and create an effective phage cocktail, it is crucial to conduct *in vivo* and in field phage therapy experiments. The ultimate goal is to apply this technology to kiwifruit orchards.

When contrasted with antibiotics and compounds based on copper, phages do have a few benefits. Natural phages are quite specific in their targeting of their bacterial host, as identified by receptors on bacterial cell walls; they spare the rest of the microbiota their attention. Their susceptibility to ultraviolet light, certain soil conditions, and the

emergence of phage-resistant bacteria are potential drawbacks to their employment.

But this may be circumvented by using phage mixtures during therapy, putting phages inside micro-and/or nanocarriers, and timing their administration to prevent exposure to UV radiation. Furthermore, in comparison to phage-susceptible bacteria, phage-resistant bacteria are both less aggressive and have slower growth rates. Combining phages with existing treatments (such as streptomycin or copper products) is another strategy to combat and prevent the emergence of microbial resistance. Another strategy is to use mutant phages that have been modified from the wild-type phage and have lytic activity again against the bacteria. Finally, another strategy is to isolate new or modified phages that are effective against the resistant bacteria.

Author Contributions

A Raza and M Hassan conceptualized the study. A Raza, M Hassan, A Khan and ZU Rehman contributed to the literature review and preparation of the original draft. S Janiad and A Riaz reviewed and edited the manuscript and provided overall supervision. A Raza, M Hassan and M Saleem worked on graphics. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

All data is available and present in the publication.

Conflict of Interest

The authors declare no conflicts of interest.

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