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Phage therapy for plant disease control with a focus on fire blight

Mini-Review

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Abstract: The concept of using bacteriophages (bacterial viruses) as biocontrol agents in pest management emerged shortly after their discovery. Although research on phage-based biopesticides temporarily stopped with the advent of antibiotics, the appearance of antibiotic resistant bacterial strains led to a renewed interest in phage therapy for control of plant diseases. In the past twenty years numerous successful experiments have been reported on bacteriophage-based biocontrol measures, and several comprehensive studies have recently been published discussing detailed results of phage application practices in pest management, mainly from North American authors. The present review focuses on bacteriophage-mediated control of fire blight (caused by *Erwinia amylovora* (Burill) Winslow *et al.*), the most devastating bacterial disease of pome fruits. Research results from North America are discussed along with recent data from European laboratories.

Keywords: *Erwinia amylovora* • *Fire blight* • *Biological control* • *Bacteriophage* • *Phage therapy*

1. Introduction

Erwinia amylovora (Burill) Winslow *et al.* is the causative agent of fire blight, the most destructive disease of several species within the family *Rosaceae*. This enterobacterial phytopathogen is present in most apple- and pear-growing regions and causes considerable economic losses in orchards [1]. The pathogen primarily infects open blossoms in the spring when warm and humid weather promotes its growth and dispersal into the vascular system of the plant [2]. Infected tissues become wilted and often necrotic, and may eventually die [3]. The disease, originating from North America, was introduced to Europe in the 1950s [4]. In Hungary it was first found in 1996 in an apple orchard, where more than 40 000 trees had to be removed because of infection by this pathogen [5].

At present, the control of fire blight is met with several difficulties, since the most effective protection method, *i.e.* the timed application of the antibiotic streptomycin to open blossoms, is banned in most European countries. Specific concerns about recently emerged streptomycin-resistant *E. amylovora* strains, along with the general trend of avoiding the use of antibiotics in agriculture, are leading to the development of alternative control strategies.

In past years, numerous studies approached this problem *via* the application of a range of promising biological control methods. These included the use of antagonistic bacterial saprophytes [6-13], plant systemic acquired resistance (SAR) inducers [14-18], and construction of transgenic plants resistant to *E. amylovora* by biotechnological methods [19-21]. Further studies on fire blight using biological control measures were directed towards the use of yeast [11] or avirulent strains of *E. amylovora* [22,23], the application of plant extracts and etheric oils [13,24,25], or the use of a new antibiotic produced by symbiotic bacteria of the entomopathogenic nematodes *Xenorhabdus budapestiensis* Lengyel *et al.* and *X. szentirmaii* Lengyel *et al.* [26]. Another novel and promising method for controlling the fire blight disease could be the use of bacteriophages.

2. Short history of phage therapy

Bacteriophages or phages are bacterial viruses that were discovered by Twort in 1915 and by d'Herelle in

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1917, who independently reported on filterable and transmissible agents of bacterial lysis [27]. In spite of the promising early results of phage therapy, the discovery of broad-spectrum activity antibiotics in the 1940s resulted in the decline of research controlling bacterial diseases with bacteriophages in the Western world [28]. However, during this period, phage therapy had been practiced mainly in human healthcare in some Eastern European countries [29,30].

In recent times, the appearance of multi-resistant bacterial strains, as well as the lack of discovering new and effective antibiotics, has resulted in a renewed interest in phage therapy in the field of medicine [29-31]. This led to the development of effective formulations like the IntestiPhage by the Eliava Institute in Georgia, which contains twenty-three different phages active against a wide range of enteric human bacteria [30].

Several factors have contributed to an increased interest in developing bacteriophage-based disease control methods in modern agriculture, such as the expanding knowledge based on successful phage applications in medicine [30,32,33], the appearance of copper and antibiotic resistant bacterial strains in the field [34], and the need for environmentally friendly pesticides. Bacteriophages were first found to be associated with plant pathogenic bacteria in 1924. Mallmann and Hemstreet later demonstrated that the filtrate of decomposed cabbage inhibited the growth of *Xanthomonas campestris* pv. *campestris* [35], and by 2005 the first phage-containing pesticide (AgriPhage™) was registered with the U.S. Environmental Protection Agency (http://www.omnilytics.com/products/agriphage). This biopesticide contains phages specifically used for control of bacterial spot and bacterial speck of tomato and pepper plants, including a mixture of wild-type phages and host range mutant phages of *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye and *Pseudomonas syringae* pv. *tomato* (Okabe) Young *et al.* [31,36, Jackson, U.S. Patent No. 4828999, 1989].

2.1 Recent use of bacteriophages against plant pathogens

Recently, bacteriophages have been found to be effective for control of several phytobacteria [37] such as *Erwinia* spp., which cause bacterial soft rot [38] and fire blight on apple and pear [23,39,40], *Xanthomonas* spp., which cause bacterial spot of tomato [41,42], peach [43,44], geranium [36], citrus [45], walnut blight [46], leaf blight of onion [47] and citrus canker [45], *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*, which causes bacterial wilt of tobacco [48], *Pseudomonas* spp., which causes bacterial blotch of mushrooms [49], and *Streptomyces scabies* Lambert & Loria, which causes potato scab [50]. In spite of this increasing research success, so far only one phage-based biopesticide (AgriPhage™) is commercially available for controlling plant pathogenic bacteria (http://www.omnilytics.com/products/agriphage).

3. Control of *Erwinia amylovora* by bacteriophages

3.1 Isolation of phages

The first step of developing a phage-based biopesticide is the isolation of bacteriophages specific to the target pathogen. Phages of *E. amylovora* may be isolated from soil surrounding the infected plant, and from the diseased plant tissue as well. Although, a number of researchers have previously isolated bacteriophages demonstrating the ability to lyse *E. amylovora* [51-53], the earliest and most complete suggestions for phage-mediated control of fire blight were made by Erskine [54], who isolated a phage from soil which lysed both an *E. amylovora* strain and a yellow saprophytic bacterium *Pantoea agglomerans* (Ewing & Fife) Gavini *et al.* (formerly *Erwinia herbicola*). Depending on whether one or multiple host bacterium isolates or species are used for phage isolation, one can obtain phages that are either host specific or display a wide host response range. Ritchie and Klos [55,56] used a single host strain for isolation of phages and reported that the tested *E. amylovora* phages isolated from aerial parts of apple trees had a narrow host range, as they were able to lyse only isolates of *E. amylovora* and the closely related saprophyte, *P. agglomerans.* On the other hand, Gill *et al.* [57] found that phages isolated using a mixture of multiple host strains had a broader host range. During our own investigations, we used several Hungarian *E. amylovora* strains for the isolation of phages (Schwarczinger and Kolozsvári Nagy, unpublished data). We found that these phage isolates have a much broader host range than well known American phage isolates (ΦEa1h ΦEa100, ΦEa104 and ΦEa116). In fact, these Hungarian phage isolates were capable of lysing not only Hungarian *E. amylovora* strains, but also those derived from other geographical areas. However, we found that other plant pathogenic bacteria distantly related to *E. amylovora* (*Xanthomonas* spp., *Pseudomonas* spp., *Agrobacterium* spp.) were not susceptible to these phages (Schwarczinger and Kolozsvári Nagy, unpublished data).

3.2 Phages combined with other microorganisms or applied as phage mixtures

Since the 1970s, more and more phages have been isolated and subsequently characterized in detail

[54-61]. However, until recently, numerous efforts to control fire blight in orchards failed because phage populations declined in the absence of *E. amylovora* [23,56,58]. This problem can be solved in two different ways, either by using avirulent *E. amylovora* strains or saprotrophic bacteria. Tharaud *et al.* [22] and Schnabel *et al.* [23] suggested the use of avirulent *E. amylovora* mutants with bacteriophages to improve phage persistence in the phyllosphere, thereby achieving reliable control efficacy, but this would carry the risk of reversion to virulence. Lehman [40] was the first to report successful application of broad host range *E. amylovora* bacteriophages in combination with phage carrier *P. agglomerans* to maintain a replicating phage population on blossom surfaces during the primary infection period, and limit the period of time that free phages were exposed to harmful sunlight [37]. Results of early studies focusing on the isolation and morphological characterization of phages within a single-phage model [51,62] allowed the establishment of successful experiments from the 1990s onwards. Schnabel *et al.* [23] demonstrated the advantage of application of phage mixtures for improving control efficacy (for a discussion see the section “Evaluation of phage effects”).

3.3 Time of treatment

The control efficacy of phages strongly depends on the timing of their application. Erskine [54] demonstrated in an experiment with pear slices that disease symptoms were prevented when *E. amylovora* and the lysogenic form of the saprophyte *P. agglomerans* were inoculated together. In another early study, Ritchie [62] observed decreased disease symptoms on apple seedlings when the ΦEa1 phage and *E. amylovora* were inoculated at the same time. Schnabel *et al.* [23] noticed that following simultaneous application of the phage mixture and the bacterial pathogen, *E. amylovora* populations were significantly reduced. In contrast, reduction of fire blight was not significant when phages were applied a day before bacterial inoculation, since phage populations remained high only in the presence of the bacterial pathogen.

3.4 Evaluation of phage effects

Effects of phages on their bacterial hosts can be studied on immature pear slices and on apple or pear blossoms by use of the so-called drop test in liquid culture. Among these, the blossom assay is the best method to select the most effective phage candidates for biocontrol, because the main strategy for controlling fire blight with biocontrol agents is preventing the accumulation of *E. amylovora* populations on nutrient-rich stigmatic surfaces of blossoms in the spring [63,64].

Therefore, Svircev *et al.* [39] and Lehman [40] used a pear blossom model to determine the control effect of bacteriophages, as well as setting up initial parameters for field experiments and selecting the best phage isolates for orchard trials. To date Lehman has evaluated most extensively the effects of bacteriophages specific to *E. amylovora.* The author [40] conducted a three-year field experiment in pear and apple orchards in Canada to study the efficacy of different phages on the blossoms of different cultivars of fruit trees. The evaluations included not only studies of the ability of phages to suppress target bacterial populations, but also monitoring population dynamic changes of both the tested phages and a selected *P. agglomerans* strain (EH 21-5) which was used as a phage carrier. Results of multiplex real time PCR monitoring showed that phages multiplied in *P. agglomerans* for two to three days after biopesticide application, though they preferred the pathogen once it was introduced into the examined ecosystem [40]. Meanwhile, on four-year-old Gala apple trees, the average population of the bacterial pathogen was significantly reduced by approximately 50% to pre-experiment epiphytic levels, exhibiting a control efficacy statistically similar to that of streptomycin. This model system for the biocontrol of *E. amylovora* has a great advantage compared to other assays involving inoculation of immature pear fruit tissue [65], because it mimics the primary host infection pathway under conditions where a phage biopesticide is expected to work [66].

Schnabel *et al.* [58] pointed to the advantages of using phage mixtures in controlling fire blight. They tested three *E. amylovora*-specific bacteriophage isolates in liquid culture, and found that individual phages (ΦEa1, ΦEa7, ΦEa116C) were slightly effective at controlling the growth of *E. amylovora* strain Ea110 in liquid culture, but the mixture of the three phages in different combinations reduced the bacterial populations by about 99% when applied at 10^4 PFU/ml. Furthermore, Schnabel *et al.* [23] inoculated apple blossoms in the field with a mixture of phages (ΦEa1, ΦEa116B, ΦEa116C), and counted healthy and symptomatic blossom clusters fifteen and twenty-two days after inoculation. There was a significant disease reduction observed on blossoms. Application of phage treatments one day after *E. amylovora* inoculation resulted in a less effective suppression of the pathogen compared to simultaneous inoculation and phage application. Applying phage treatments one day after Ea110 inoculation, the number of *E. amylovora*-infected blossom clusters was reduced by 26% and 17.2% at fifteen and twenty-two days after inoculation, respectively. However, applying Ea110 and the phage mixture together, the incidence of fire blight

was reduced by 37% and 31% at fifteen and twenty-two days after inoculation, respectively.

Schwarczinger *et al.* [67] used the blossom assay as well. They wanted to know whether the elimination effect of phages on *E. amylovora* populations depends on the test plants' susceptibility to the pathogen. They found that the two selected bacteriophage isolates significantly reduced the number of bacteria re-isolated from flowers by at least 45% compared to the controls on all of the three investigated apple cultivars displaying different susceptibility to *E. amylovora* (Figure 1). The best results were obtained on the moderately resistant apple cultivar Freedom, where the phage H6 reduced the number of re-isolated bacteria by 90%. It can be concluded that the bacteriophages used in this study (H6 and H5B) are highly efficient in eliminating *E. amylovora* on apple flowers, especially in a moderately resistant apple cultivar under *in vitro* conditions.

Schwarczinger and Kolozsvári Nagy (unpublished data) studied the biocontrol effects of Hungarian phage isolates in mixture on four ornamental plants (*Pyracantha angustifolia* (Franch.) Schneid., *Cotoneaster horizontalis* Decne., *Sorbus domestica* L. and *Crataegus monogyna* Jacq., Figure 2). For all four plant species, the application of phages in mixture provided a better biocontrol effect than individual applications; however, in most cases there were no significant differences in efficacy between the most effective phage (Φ-EaH2A) and the phage cocktail (Schwarczinger and Nagy, unpublished data). The best suppression effect was obtained on phage cocktail-treated *P. angustifolia* where necrosis was not observed on flowers even four days after inoculation. In contrast, treatment with the phage mixture did not significantly reduce flower necrosis on *S. domestica* and *C. monogyna* (reduction of symptoms ranged from 12% and 28%). Infection rates of untreated controls indicated that the treated *Sorbus* and *Crataegus* species are much more susceptible to *E. amylovora* than the *Pyracantha*, and the *Cotoneaster* plants. Such an increased susceptibility to bacterial infection could explain the inefficiency of phages on the plant species mentioned above.

It is extremely difficult to compare results of independent experiments on the effect of bacteriophages on *E. amylovora.* These experiments differ in several parameters including phage concentrations, the timing of bacterial inoculations, and phage treatments or application of phages either alone or in mixture with or without carrier microorganisms. Furthermore, in most published experiments no positive controls, *i.e.* streptomycin or commercial biological control products, have been used. Table 1 summarizes the suppressive effects of several biocontrol agents on *E. amylovora* infection. Based on the presented data it is obvious that the efficacy of commercialized, bacteria-based biocontrol agents is lower than that of streptomycin. However, when the efficacy of the given biocontrol agent exceeded 55%, no streptomycin control was included (based on data of Table 1). According to our knowledge only Lehman [40] reported on studies where the efficacy of different phage + carrier (*P. agglomerans* EH21-5) combinations was compared to that of other commercial biocontrol products (BlightBan®A506,

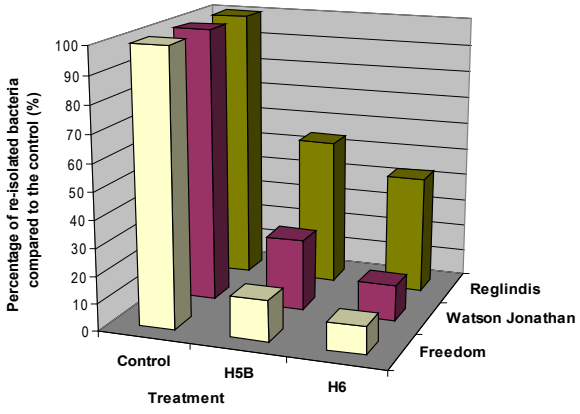

Figure 1. Effect of phages on *Erwinia amylovora* infection on flowers of different apple (*Malus domestica* Borkh.) cultivars. Hungarian phage isolates (H5B, H6) applied in spray inoculation on flowers (10^{10} PFU/ml), significantly reduced the number of re-isolated bacteria on all three apple cultivars tested, compared to the untreated control. However, a significant difference was not detectable between the effects of the two phages (data of significant differences not shown) [67].

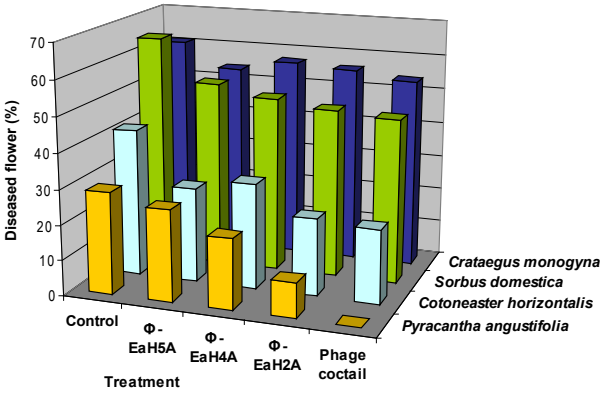

Figure 2. Disease control by bacteriophages on flowers of some ornamental plants infected with *Erwinia amylovora*. Plants (100 flowers/plant) were sprayed by Hungarian phage isolates (Φ-EaH2A, Φ-EaH4A, Φ-EaH5A) individually (10^4 PFU/ml) or in combination, and 20 seconds after phage treatments they were inoculated by a bacterial suspension of *E. amylovora* strain Ea 1/79 Sm (10^9 CFU/ml). Suppression of *E. amylovora* was evaluated by assessing necrosis of flower ovaries four days after inoculation (Schwarczinger and Kolozsvári Nagy, unpublished data).

Biological control agent	Mean % average of disease reduction		Reference
	biological control agent	streptomycin	
Pantoea vagans C9-R1[a]	17.0-78.0	65-89	[68]
Pantoea agglomerans Eh252	55	75	[69]
BlightBan®A506	12,5	61.0	
BlightBan®C9-1	33.1	63.3	[70]
Bloomtime Biological™ FD Biopesticide	28.5	67.3	
Pantoea agglomerans HIP32	46[f]	68	[12]
ΦEa1, ΦEa116B, ΦEa116C in mixture	37.0[b] 36.1[c]	NT	[23]
Pantoea agglomerans EH 21-5+ ΦEa21-4	50[d]	63[d]	[40]
Pantoea agglomerans EH 21-5+ ΦEa46-1			
ERWIPHAGE Patent number P0700600	71-75[e]	NT	(T. Kovács, personal communication)
H5B	85[f]	NT	[67]
H6	90[f]	NT	

Table 1. Examples of control of *Erwinia amylovora* in blossoms by biocontrol agents and streptomycin. NT not tested, [a] Formerly *Pantoea agglomerans*, [b] Inoculation of *E. amylovora* and phage mixture was carried out on the same day, [c] *E. amylovora* inoculated one day before the application of phage mixture, [d] approximate data, exact data was not available, [e] results were evaluated three and five weeks after treatments, [f] tested only during *in vitro* conditions.

BlightBan® C9-1). The author observed that two phage + carrier combinations (ΦEa21-4 + EH 21-5 and ΦEa46-1 + EH 21-5) and the streptomycin control had similarly reduced the incidence of *E. amylovora* by 50%, and 63%, respectively. However, it is worth mentioning that the application of BlightBan® C9-1 by itself gave a similar result.

3.5 Molecular characterization of phages

As mentioned above, one of the main hurdles of successfully controlling bacterial diseases with bacteriophages is the risk of evolution of phage-resistant bacterium strains. Roach *et al.* [71] studied phage resistance by Dualplex real-time PCR to find out whether it is induced by the development of prophages or mutations within the bacterium. Prophages were detected in twenty-four of the twenty-seven phage-resistant Ea110 isolates, thus lysogeny was responsible for their resistance. On the contrary, there were no detectable prophages in any of the six Ea29-7 phage-resistant isolates; therefore, in this case resistance was thought to be the result of bacterial mutation. Based on early studies [54,55] it has been shown that the virulence of *E. amylovora* is attenuated in phage-resistant bacteria. A similar phenomenon was also reported for *Pseudomonas morsprunorum* (Wormald) Yong *et al.* [72]. Roach *et al.* evaluated the role of bacterial exopolysaccharides (EPS) in phage resistance [73]. The authors found that increased EPS production led to higher propagation rates of bacteriophage populations, while mutants deficient in amylovoran were resistant to bacteriophage attack. The results discussed above point to the importance of clarifying the genetic background of these bacterial viruses. To date, several comprehensive studies have been carried out that focus on the molecular characterization of a wide range of *E. amylovora* bacteriophage isolates. The first such study evaluating the diversity of *E. amylovora* phages collected from soil samples and shoots of fire blight-infected apple, pear, and raspberry tissues was conducted by Schnabel and Jones [58]. Based on the detailed molecular characterization of fifty phage isolates, five distinct phages, including relatives of ΦEa1 and ΦEa7 as well as three novel phages, were identified. The authors found that these phages were highly specific to *E. amylovora*. In 2003, Gill *et al.* [57] estimated the diversity of bacteriophages collected from orchards in southern Ontario and reported the detailed characteristics of various sets of phages with broad host ranges. Forty-two phage isolates were identified within six distinct phage types based on molecular characterization of the phages using a combination of PCR and restriction endonuclease digestions.

Despite the extensive research done until 2009, sequence information of *E. amylovora* phages was limited to the genome of Era103 (GenBank accession number NC_009014) and a 3.3-kb region of the ΦEa1h genome [74]. Lehman *et al.* [59] described the first complete genome sequence for a myoviridal bacteriophage ΦEa21-4 (GenBank accession number NC_011811).

This phage, infecting *E. amylovora*, *E. pyrifoliae* Kim *et al.* and *P. agglomerans* strains, was previously isolated from soil under a pear tree showing fire blight symptoms [57]. The ΦEa21-4 phage, like those isolated by Gill *et al.* [40], has a very broad host range and has shown great potential to become a biopesticide, but has not been detected so far by PCR [40]. Lehman *et al.* [59] suggested that sequence analysis would likely enhance the development of tests for monitoring populations of ΦEa21-4 and related phages in orchards. Recently, Born *et al.* [75] found that none of the twenty-four tested novel *E. amylovora*-specific phages originating from Switzerland contained a lysogeny control region in their genome, indicating strictly lytic life-cycles. Müller *et al.* [76] studied properties of *E. amylovora* bacteriophages collected from North America and Germany and found that phages ΦEa104 and ΦEa116 reduced fire blight symptoms on flowers and immature pears significantly better than ΦEa1h and ΦEa100. Based on their PCR analysis results using primers specific for American phages, they found that the phages from Germany isolated by Müller and co-workers seem to be different from the North American bacteriophages. Müller *et al.* [77] described the genome sequence of three *E. amylovora* phages from North America (ΦEa1h, ΦEa100, ΦEa104), and a novel phage, ΦEt88, that was isolated as a prophage of *E. tasmaniensis* Geider *et al*., an antagonistic bacterium for *E. amylovora* from Australia (nucleotide sequences are available in the EMBL database under accession numbers FQ482084, FQ482086, FQ482083 and FQ482085).

4. Conclusions and future prospects

Due to the considerable amount of information on the biology and ecology of bacteriophages that infect plant pathogens [51,52,78-80, Gill and Abendon, Bacteriophage ecology and plants, APSnet http://www.apsnet.org/publications/apsnetfeatures/Pages/BacteriophageEcology.aspx], including molecular characterization of these phages [57-59], it has been revealed that bacteriophages have many characteristics that make them attractive as potential biological control agents in agriculture [31,81]. On the other hand, they have a lot of disadvantages which could generate several difficulties in agricultural phage therapy applications. Since numerous extensive reviews on this subject have been published in past years [31,37,66], a comprehensive review of advantages and disadvantages of phage therapy is beyond the scope of this paper. Therefore, we emphasize only the most important aspects of this problem (Table 2-3).

Phages are self-replicating and self-limiting, they can be targeted against bacterial receptors that are essential for pathogenesis, they are abundant in nature (showing a wide diversity), and they appear to be non-toxic to the eukaryotic cell [82,83]. Due to the specificity and the narrow spectrum of phage activity, bacteriophages can be very selective on the bacterial populations they attack, reducing the likelihood of damaging other, possibly beneficial members of the native flora [81,37]. Their isolation, production, and storage are relatively easy and inexpensive [31,84].

Besides these attractive features, the known disadvantages of phages as biopesticides documented in the literature are as follows: the potential for alteration of phages from virulent to temperate, the development of phage-resistant hosts, and the complexity of implementing biological control measures under different environmental conditions [31,51,85]. The latter problem is an especially difficult challenge given that phages can be inactivated by heat, pH extremes, desiccation, UVA and UVB irradiation, exposure to certain chemical pesticides such as copper compounds, or washed off from surfaces by rain [41,82,86]. Consequently, the harsh phyllosphere environment is not ideal for phage survival and therefore results in the loss of phage viability unless they are protected during treatments [41,43,58,86-88]. The lack of standardization of phage preparations and the lack of criteria for purity and efficacy makes it extremely difficult to compare most of the studies that had been published. A further disadvantage of phage therapy applications is the fact that registration of biocontrol agents is usually a long, difficult, and expensive process [89]. However, the main reason for the perception that phage therapy field applications are futile is probably due to the frequent observation of phage resistance. Despite promising early results, phage therapy did not prove to be a reliable and effective method for plant disease control, and was deemed unfeasible by several leaders in the field [85,90].

Many of the challenges of applying phages as biopesticides can be resolved by using resistance management techniques such as avoiding the employment of phages that are capable of displaying lysogeny, a delayed infection during which the temperate phage genome usually integrates as a prophage into the host bacterial chromosome (Gill and Abendon, Bacteriophage ecology and plants, APSnet, http://www.apsnet.org/publications/apsnetfeatures/Pages/BacteriophageEcology.aspx). In fact, phages that have a lytic life cycle, as opposed to lysogeny, are the ideal biopesticide candidates. During infection by lytic phages, bacterial cells are lysed and destroyed

Characteristics	Notes
Self-replicating	
Self-amplifying	- during phage treatments host cells serve as sustained reservoirs for new phage infections while the shortage of host bacteria leads to the decline of phage populations [95,96]
Self limiting	
Wide diversity	- phages are common in nature and estimated to be the most widely distributed and diverse organisms in the biosphere [78,95]
Inexpensive	- their isolation, production and storage are quite easy and inexpensive [31,84] - phages can be isolated from wherever bacteria are present (soil, natural water, sewage, plants, animals and the human body) [78]
Narrow host range - High specificity	- they can be targeted against bacterial receptors functional in pathogenesis [95] - because of high host specificity of bacteriophages they are harmless to beneficial members of the native flora [37,81]
No hazard to eukaryotic cells	- some investigators consider phages to be non-toxic to eukaryotes [83] while others emphasize the risk of toxicity [97,98]
Application in integrated pest management	- phage treatments may be combined with pesticide applications [99]

Table 2. Main advantages of phage therapy in pest management.

Characteristics	Notes	Strategies
Potential for alteration from virulent to temperate	- development of phage-resistant host bacterial strains [51,85]	- application of various phages that have different cell surface receptors in a mixture overcome the risk of reduction in efficacy due to bacterial resistance [71,73] - application of a mixture of host-range mutant (h-mutant) phages may increase the control efficacy [42] - selection of lytic phages and clarifying the inclination of phages to transform into prophages [75] is very important during preliminary studies
	- bacteriophages may transduce various characters (e.g. genes encoding virulence factors or toxins) from one host to another, and can introduce active prokaryotic genes into plant and animal cells [97,98] and transform harmless commensal bacteria into pathogens [100]	
High sensitivity to different environmental conditions (*e. g.* UVA, UVB, desiccation, temperature, pH, chemicals)	- protective or carrier formulations can increase persistence of phages on the treated area	- formulations consist of milk, sugar, or flour [31,41,51,86-88] - application of non-pathogenic or antagonistic bacteria as phage carriers [39]
	- proper timing of application	- evening and dawn phage applications attenuated the sunlight effect resulting in increased persistence of phage populations and improved disease control [101] - time of phage application relative to the bacterial pathogen [23,43,54,62]
Relatively low control efficacy and consistency	- level and consistency of control measures with phages is lower than those with antibiotics [58,64]	- control effect can be increased by *e.g.* application of phages in combination with other biocontrol agents

Table 3. Main disadvantages of phage therapy in pest management.

after immediate replication of the virion. It has been demonstrated that by application of lytic phages in a phage cocktail [41,42,45,49,91], control efficacy can be significantly improved [66]. Furthermore, by applying a mixture of host-range mutant (h-mutant) phages, the control effect can be increased even further [42]. Jackson developed a new, patented process that involved preparing a mixture of h-mutants, phages possessing the ability of lysing bacterial strains resistant to the parent phage, while still being capable of lysing the wild type bacterium (Jackson, U.S. Patent No. 4828999, 1989). Thus, they have an extended host range compared to the parent phage. Implementing this kind of phage application twice a week, early in the morning prior to sunrise, provided a significantly more effective form of disease control of tomato bacterial spot than the standard copper-mancozeb treatment [42]. Strategies to improve phage persistence in the hostile plant leaf surface environment include protective carrier formulations [86-88] consisting mainly of milk, sugar, and flour [41], adequate phage concentration, proper frequency and timing of application relative to the appearance of the bacterial pathogen [23,43], and the avoidance of sunlight. A further pivotal strategy to improve phage persistence is the establishment of a non-pathogenic bacterial host population in the phyllosphere for maintaining phage

populations, as well as potentially serving as biocontrol agents due to the antagonistic effects these bacterial strains usually have [39].

At present, control of *E. amylovora* with bacteriophages is under investigation mainly in Canada and in the United States of America. Studies in Europe are currently limited to only a few countries with a primary focus on the morphology, molecular characterization, and host range studies of *E. amylovora* phages and examination of phage efficacy during *in vitro* conditions [61,67,74-77]. Apart from some exceptions, these investigations do not report any field experiments with these phages. So far there are no patented phage-based biopesticides effective against fire blight, but one that is currently under patent processing in Hungary, Erwiphage, contains different *E. amylovora* phage isolates in a special UV protective formula (registration number: P07 00 600) and seems to have a very promising protective effect due to the fact that it resulted in 71-75% reduction of disease incidence on Jonagold apple trees in field experiments (T. Kovács, personal communication). The prospective practical application of phage-based biopesticides to control fire blight requires developing measures that improve environmental persistence of phages.

Studies of bacteriophages of plant pathogenic bacteria have dealt primarily with their use as a diagnostic tool [92], or in characterization of phage-bacteria interactions [51,52,93]. At present, a considerable amount of research data has accumulated on the biology and ecology of bacteriophages of several plant pathogens, including the molecular characterization of phages. Recently, phages are being used not only in human and veterinary healthcare, food industry, genetics, and diagnostics, but also in pest management. Based on successful medical applications of microencapsulated bacteriophages [94], this kind of physical protection of phages is suggested for use in pest management as a promising future technology to enhance phage endurance in orchards [66]. Assessment of different phage mixtures in order to achieve an improved control efficacy, or the selection of suitable epiphyte bacterial isolates as carriers of phages is also emphasized. Application of phages for controlling plant pathogens could be especially promising where it can be used with other control methods, for example with antagonistic microorganisms, or on moderately resistant plants. Due to constantly changing environmental conditions in the phyllosphere of orchards, it is and likely will continue to be difficult to obtain a reliable control efficacy without the contribution of phage-based biocontrol measures used as part of integrated pest management strategies.

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