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Phages of phytopathogenic bacteria: High potential, but challenging application

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Abstract: Phytopathogenic bacteria are one of the most significant causes of crop yield losses. Until now, the direct treatment of bacterioses was limited to the application of antibacterial compounds or resistance inducers. This is about to change due to the revolutionary discovery of phages. Indeed, bacteriophages look very promising as therapy agents: cheap, self-amplifying, self-eliminating, and safe for the host organism. However, phage therapy of plant diseases remains a "direction with high potential", which, so far, has very few successful implication cases. Here, we discuss recent advances in phage research, focusing on the challenges associated with the evaluation of phage biological activity, under both laboratory and environmental conditions.

Keywords: bacteriophages; field application; plant protection; phage application

A growing demand for products of "organic quality" together with strict limitations of genome editing in food production make the treatment of plant diseases a non-trivial challenge (Yin & Qiu 2019). Bacteriophages (phages) appeared at the beginning of the 20th century as promising antibacterial agents to treat plant diseases. Due to their high specificity, no negative impact on humans or animals, and no environmental pollution or residue, phage therapy is considered as one of the most favourable methods in replacing chemical controls in agriculture (Vu & Oh 2020). Nowadays, hundreds of bacteriophages have been isolated and tested for their ability to inhibit the development of pathogenic bacteria in plants, and there are still more phages waiting to be discovered. Although several bacteriophages have been reported to be effective in the laboratory and in field trials, the number of practically approved preparations is still insufficient for both animal and plant pathogens (Kassa 2021). In this review, we aim to draw attention to the challenges in the preparation of new phage-based products for the biocontrol of plant diseases, especially in the evaluation of their efficiency in laboratory conditions and in field trials.

Phages as a tool for the biocontrol of plant pathogens. Phage research targets the most abundant and devastating plant pathogens. In recent years, phages have been isolated from bacteria infecting the majority of crops, especially those causing post-harvest yield losses in potato, cabbage and carrot crops. Indeed, isolated lytic bacteriophages

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specific to *Pectobacterium carotovorum* subsp. *carotovorum*, *P. wasabiae* and *Dickeya solani*, significantly reduced the soft rot infections on potato tubers by at least 80% in comparison to the controls inoculated with a mixture of bacteria only (Czajkowski et al. 2014; 2015). T4-like phage cocktails were effective against *Dickeya solani*, as seen by the reduced disease incidence and severity on potato tubers in laboratory assays (Adriaenssens et al. 2012). *Pectobacterium atrosepticum* phages were successfully used in preventing the rotting of harvested potato tubers (Carstens et al. 2019; Zaczek-Moczydłowska et al. 2020). In general, phages or phage cocktails have been proven efficient when applied at the post-harvest stage. In this setup, vegetables are sprayed/washed in solutions containing phages, creating a protective film on the vegetable surface, while no interactions between the plant parts and phages have been described.

Protecting plants from bacterioses during vegetation using phage applications appears more challenging, though not impossible. Interestingly, the majority of phages used for the biocontrol are tailed phages (mostly belonging to *Podoviridae* or *Myoviridae*), while the use of filamentous phages has high potential as well (Sharma et al. 2019). For example, phages of *Pectobacterium odoriferum*, a destructive pathogen causing soft-rot disease in various vegetables, suppressed bacterial growth in the seedlings of Kimchi cabbage in a greenhouse (Lee et al. 2021). The spraying of lettuce leaves with a suspension of phages of *Pectobacterium carotovorum* subsp. *carotovorum* decreased the percentage of diseased plants (Lim 2013). Also, the spraying of specific phages isolated from *Erwinia amylovora* on opened apple flowers decreased the appearance of disease symptoms (Boulé et al. 2011). Phages specific to a potato pathogen, for example, *Streptomyces scabies*, significantly reduced the lesion coverage in the treated seed tubers, with no significant impact on the biomass, size or number of plants grown from those tubers (McKenna et al. 2001). Another experiment with the *S. scabies* phages showed a significantly reduced weight loss in the infected plants (Goyer 2005).

Phage biocontrols have successfully been applied to a number of crop pathogens in both greenhouse and field conditions. This includes pathogens from the genera *Xanthomonas* (bacterial spot of tomatoes, peaches, geraniums and citruses, onion-blight, walnut blight, and citrus canker) (Lang et al. 2007; Gašić et al. 2018), *Ralstonia* (bacterial wilt of tobacco) (Fujiwara et al. 2011; Addy et al. 2012; Ramírez et al. 2020; Umrao et al. 2021), *Erwinia* (fire blight, bacterial soft rot) (Ravensdale et al. 2007; Boulé et al. 2011; Frampton et al. 2012; Nagy et al. 2012; Park et al. 2018), *Agrobacterium* (crown gall of tomatoes) (Zimmerer et al. 1966; Stonier et al. 1967) and *Pseudomonas* (bacterial speck of tomatoes, bacterial blotch of mushrooms) (Kim 2011; Rombouts et al. 2016; Rabiey et al. 2020; Akbaba & Ozaktan 2021). Several examples have recently been documented, as *Xanthomonas campestris* pv. *vesicatoria* phages in the treatment of tomato plants (Obradovic et al. 2004), or specific phage therapy against *X. oryzae* in rice (Ranjani et al. 2018). Also, phages of *X. axonopodis* pv. *allii* lysed a significant part of bacterial load in infected onion plants (Lang et al. 2007), and phages specific to *X. axonopodis* pv. *citri* decreased the disease incidence and severity of symptoms in pre-treated grapefruit plants (Balogh et al. 2008). Moreover, phages of *Pseudomonas syringae* pv. *porri* reduced the length of lesions associated with bacterial rot in leek plants (Rombouts et al. 2016). Phages of *P. tolaasii* were found to be efficient against the brown blotch disease of oyster mushrooms (Kim 2011). Promising results of *P. syringae* pv. *syringae* biocontrols by specific phages were obtained in preventing the disease in cherry plants (Rabiey et al. 2020; Akbaba & Ozaktan 2021). A propagation of *P. syringae* pv. *actinidiae*, a kiwifruit pathogen affecting orchards, has been successfully reduced with phages directly inoculated into the bacteria infected leaves (Lallo et al. 2014; Pinheiro et al. 2020; Song et al. 2021). The tomato pathogen *P. syringae* pv. *tomato*, known also as a model pathogen of *Arabidopsis thaliana*, is another important target for phage hunters. For instance, the phages FRS (*Podoviridae*) and SHL (*Myoviridae*) significantly reduced the final disease symptoms caused by the spray-inoculation of *P. syringae* pv. *tomato* in tomato leaves (Morella et al. 2018) and the disease progression in flood-inoculated tomato seedlings (Hernandez et al. 2020). The aforementioned successful experiments prove the effectiveness of bacteriophages, but a large number of inoculation methods and processing results have complicated the process of phage therapy in being released for wider use.

Variety of phage application protocols. The common strategy for the preparation of com-

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mercial phage products also called "bactericides based on phage technology" (Moye et al. 2018) includes the following steps: (1) phage isolation from the natural environment, likely from the material where the host bacteria actively grow, (i.e., rotten vegetables or waste water); (2) phage characterisation (morphology, biochemical properties, host range, genome sequencing, proteome analysis, phylogeny); (3) antibacterial tests *in vitro* and *in vivo*; (4) optimisation of the phage cocktail for the biocontrol (i.e., field trials); (5) patenting. The majority of studies are held in laboratory or greenhouse conditions, while true field trials remain less explored. While the first stages of phage preparation tests are predominantly standardised, biological tests are highly inconsistent between studies (summarised in Figure 1). When it comes to the field application trials, phage products have been either used in a form of a seed coating (Kimmelshue et al. 2019), added to the soil as a suspension, or applied on vegetating plants in spray form of (Morella et al. 2018; Rabiey et al. 2020; Akbaba & Ozaktan 2021). In the case of greenhouse experiments, phages have been applied either prior to or simultaneously with the inoculation against the target pathogen. In field trials, treated plants are cultivated along with the control group, and the infection happens naturally. Different ways of phage applications are needed to deal with the potential decrease in the phage virulence due to environmental factors (UV light, desiccation, chemical destruction by soil compounds/enzymes) (Balogh et al. 2010) (Figure 1, left panel). To overcome these factors, phage suspensions can either be pre-mixed with protecting agents like polyvinylpyrrolidone or polyvinyl alcohol before drying in a form of a film on the seed surface (Kimmelshue et al. 2019) or applied at certain time points with favourable environmental conditions (i.e., at dusk, to ensure high humidity and low light intensity during the first

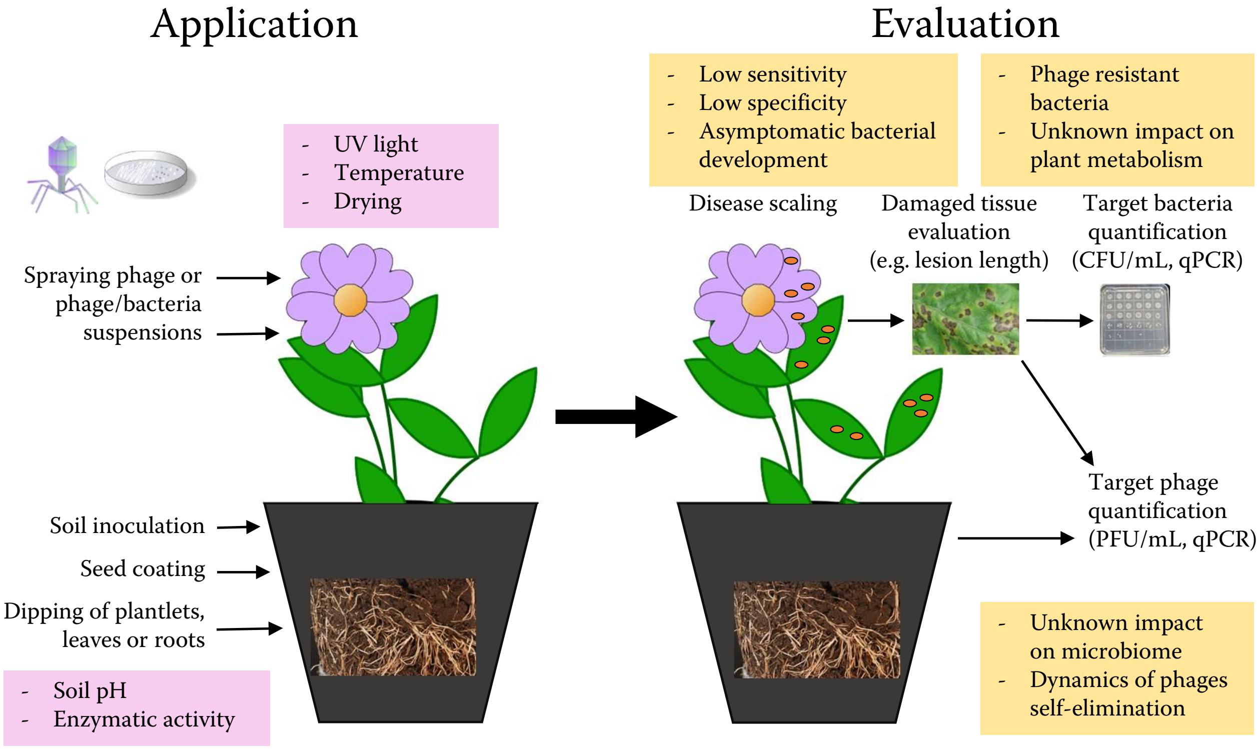

Figure 1. A schematic overview of phage efficiency studies

The phage application is performed by either spraying phage particles on the plant's aerial parts (leaves, sprouts or blossoms) or by adding phage preparations to the soil (inoculation with watering, seed coating or dipping of plant parts before planting). The pink boxes indicate environmental factors limiting the phage efficiency. The evaluation of the phage efficiency starts with scaling the disease symptoms, mostly by custom scales designed for the patho-system. The next evaluation step is the quantitative measurement of the damage (i.e., lesion length, macerated tissue weight), followed by the quantification of the bacterial load and/or quantification of the phage load in the plant tissues or soil. The yellow boxes indicate any method limitations and plant health defining factors that are often overlooked hours of the phage action) (Obradovic et al. 2004). After the phage application and exposure to disease causing agents, plants are cultivated normally until the disease symptoms have developed to evaluate the phage efficiency.

A striking number of new phages with a high biocontrol potential have been reported in recent years, increasing the need for unification of efficiency testing protocols. So far, distinct approaches and methods have been used to model the potential of these phages in laboratory conditions and field trials, however, no standard protocol is accepted in evaluating the efficiency of the phage treatment. Some studies are based on the visual assessment of the symptoms, others focus on the plant biomass, while only rare works have taken the bacterial and/or phage quantification in the issues into account (summarised in Table 1). Studies focusing on the post-harvest storage of crops (e.g., potatoes or carrots) mostly rely on custom visual scales to evaluate the phage efficiency (Fujiwara et al. 2011; Addy et al. 2012; Umrao et al. 2021). This approach is indeed very convenient due to low expenses and adjustability; however, it is difficult to extrapolate the results on other experiments or to compare the product efficacy in other trials. As the big aim is to investigate whether certain phages are good candidates for use in phage therapy, it seems legitimate to rely on the development of symptoms, the plant biomass and plant product quality. Nevertheless, with this approach, the possibility of asymptomatic bacterial development cannot be excluded, which might lead to only temporary effects of the phage products. Another, more precise, method is the direct quantification of plaque forming units), and normalisation of the number of phage particles to the biomass of treated plant tissues, as used in the treatment of *Ralstonia solanacearum* infected banana plants (Ramírez et al. 2020), the prophylactic inoculation of rice seedlings with *Xanthomonas oryzae* phages (Ranjani et al. 2018) or the treatment of an *Xanthomonas axonopodis* pv. *allii* infection on onions (Lang et al. 2007). The most precise (though rarely applied) approach is the quantification of the phage DNA by quantitative polymerase chain reaction (qPCR) (Das et al. 2015) and normalising it to the number of bacteria measured in colony forming units. This method could be a better option than just visual diagnostics or bacteria and phage titre calculations; shedding light on other aspects of the interaction between phages, bacteria and plants, which we will discuss further.

The unknown role of phages in the phyllosphere. Limited research has been undertaken on the phage impact on microbial communities, and most often, such research has only been concerned with the soil microbiome (Williamson et al. 2017). Indeed, rhizosphere phages have the potential to modulate the soil bacterial community structure and organic matter cycling (Pratama et al. 2020). Phages may influence the plant nutrient availability through potential effects on the soil trophic network by driving mutations or regulating the gene expression in selected bacterial phyla (O'Brien et al. 2019; Sharma et al. 2019; Starr et al. 2019). A cocktail of five phages was sufficient to affect and modify a bacterial community; not only when inoculated in a sterile soil during and after the soil colonisation with bacteria, but also in natural soil (Braga et al. 2020). The phage addition affects the microbe-mediated chemical processes in the soil, such as N-cycling. It could be due to the lysis of the host cells by the phages, resulting in a release of organic nitrogen followed by its mineralisation. To study the behaviour of phages in the context of the phyllosphere, Morella et al. (2018) transferred microbial communities from field-grown tomato plants to juvenile plants grown mostly under sterile conditions in the presence or absence of the associated phage community (Morella et al. 2018). In these experiments, tomato leaves were spray-inoculated with phage suspensions or phage-bacteria mixes, and the compositions of the leaf-associated microbiome were analysed. Across the experiments, the authors observed a decrease in the overall bacterial abundance 24 h after the spray-inoculation, suggesting that phages affected the growth of the most common and/or fastest growing bacterial strains during colonisation of a new plant host. However, a general decrease in the bacterial abundance was not necessarily the expected outcome of lytic phages. On the contrary, phage-mediated lysis could increase the total bacterial population growth due to the release of nutrients (Brockhurst et al. 2006; Weitz & Wilhelm 2012).

A simplified vision of the direct effect of phages on the microbiome is killing target bacteria and leaving space for phage resistant groups (Brockhurst et al. 2006; Koskella & Brockhurst 2014). However, it is unclear, whether the ecological and evolutionary processes of phage resistance

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Table 1. Phages reported in 2001–2021 with the potential to be biocontrol agents

Pathogen	Crop	Phage application procedure	Efficiency evaluation	Reference
Pectobacterium carotovorum subsp. *carotovorum*, *P. wasabiae*, *Dickeya solani*	*Solanum tuberosum* (potato)	Potato tubers were cut into transverse disks with wells and filled with a mixture of one of the tested bacteriophages together with each bacterial strain tested	Ratio of the average diameter of rotten potato tissue around the wells co-inoculated with bacteria and bacteriophage to the average diameter of rotten tissue around wells inoculated with bacterial mixture only	Czajkowski et al. 2015
Dickeya solani	*Solanum tuberosum* (potato)	Phages LIMEstone1 and LIMEstone2 were added to tubers inoculated with LMG 25865	Weight of the tuber before treatment and after the rotten tissue was scraped off	Adriaenssens et al. 2012
Dickeya solani	*Solanum tuberosum* (potato)	A potato slice assay, where potato tubers were cut into disks with wells and inoculated with phage and bacterial suspensions	Ratio of the average diameter of rotten potato tissue around wells co-inoculated with bacteria and bacteriophages to the average diameter of rotten tissue around wells of the positive control	Czajkowski et al. 2014
Erwinia amylovora	*Malus domestica* (apple) and *Pyrus* L. (pear)	Phage suspensions spraying. Blossoms were then dried for 3 h before inoculation with a bacteria suspension	Disease symptoms scored four to five days after inoculation	Boulé et al. 2011
Xanthomonas oryzae	*Oryza sativa* (rice)	Rice seeds were immersed in broth containing bacteria and incubated overnight before treatment with suspension of phages	Homogenates of seeds samples were centrifuged; pellet was used to determine a number of bacteria after phage treatment, supernatant was taken to determine a number of phage particles by spot-test	Ranjani et al. 2018
Streptomyces scabies	*Solanum tuberosum* (potato)	Phage bath with valve-controlled air-lift was constructed by authors	Scab lesions per potato tuber were counted and severity assessed as percentage lesion surface area and lesion type using a custom scale	McKenna et al. 2001
Ralstonia solanacearum	*Solanum lycopersicum* (tomato)	Plants were planted in peat pellets pre-soaked in phage suspension. Two days later, plants were cut at the root tips and dipped in a bacterial suspension	Symptoms of wilting graded according to a custom scale	Fujiwara et al. 2011
Xanthomonas campestris pv. *vesicatoria*	*Solanum lycopersicum* (tomato)	Prior to setting into the field, tomato transplants were inoculated by spraying the *X. campestris* pv. *vesicatoria* inoculum and after transplanting they were sprayed with phage suspension twice per week in evening	Disease severity assessment using the Horsfall-Barratt scale, measuring the area under the disease progress curve, and assessment of lesion numbers on terminal leaflets	Obradovic et al. 2004
Xylella fastidiosa subsp. *fastidiosa*	*Vitis* sp. (grapevine)	Individual plants were inoculated between the second and the third node on opposite sites (two points/plant) with bacterial suspension and phages using the needle inoculation technique	Tissue extracts assayed for phage and *Xf* housekeeping genes by qRT-PCR	Das et al. 2015
Xanthomonas axonopodis pv. *allii*	*Allium cepa* (onion)	Phage suspension spraying	Phage propagation and calculation of plaques	Lang et al. 2007a
Pectobacterium carotovorum subsp. *carotovorum*	*Lactuca sativa* (lettuce)	Phage suspension spraying	Percentage of diseased plants and visual differences in growth	Lim 2013

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Table 1 to be continued

Pathogen	Crop	Phage application procedure	Efficiency evaluation	Reference
Streptomyces scabies	*Raphanus raphanistrum* (radish)	Seeds were incubated in tubes containing water agar, and then bacterial and phage suspensions were added	Percentage of weight loss	Goyer 2005
Xanthomonas axonopodis pv. *citri*	*Citrus* × *paradisi* (grapefruit)	Greenhouse trials: phages were applied either in sterilized tap water or in a suspension of skim milk powder. ACC nursery trials: a locally isolated bacteriophage was sprayed using a hand pressurized backpack sprayer	Disease incidence calculated as the percentage of diseased plants, severity was estimated using the Horsfall-Barratt (HB) scale. Disease intensity determined by collecting all diseased leaves from a plot, counting the total number of lesions, and calculating the average lesion number per leaf	Balogh et al. 2008
Pseudomonas syringae pv. *porri*	*Allium ampeloprasum* L. (leek)	*In planta* activity of the phages was tested by injecting phage and bacterial suspensions into leek leaves	Lesion lengths, disease incidence (number of damaged plants) and disease severity (% of leaf surface affected)	Rombouts et al. 2016
Pseudomonas tolaasii	*Pleurotus ostreatus* (oyster mushroom)	Phage lysates were mixed with bacteria at a 1 : 1 ratio and mixture was dropped on the surface of the mushrooms	Measuring the size of the blotches	Kim 2011
Pseudomonas syringae pv. *syringae*, *P. syringae* pv. *morsprunorum*	*Prunus cerasus* (cherry)	Cherry leaves were sprayed with bacteria either alone (control) or with phages (phage cocktails)	Quantification of bacterial colonies	Rabiey et al. 2020
Ralstonia solanacearum	*Musa acuminata* (banana, plantain)	Suspension of phages and bacteria was added to the soil where banana plants were growing	Concentrations of bacteria and phages were calculated from serial dilutions of the soil microcosms using the double agar layer and the spot plaque method	Ramírez et al. 2020
Pseudomonas syringae pv. *syringae*	*Prunus avium* (sweet cherry)	Bacteria were sprayed onto cherry plantlets during micropropagation, kept in the dark for 12 h and sprayed with phage suspension	Symptoms were evaluated 10 days after pathogen inoculation using the 0–9 disease severity scale	Akbaba and Ozaktan 2021
Pseudomonas syringae pv. *actinidiae*	*Actinidia deliciosa* (kiwifruit)	Kiwifruit leaves were cut and immersed in bacteria and phage suspensions	Phage titre was determined by double-layer agar method	Pinheiro et al. 2020
Pseudomonas syringae pv. *tomato* DC3000	*Solanum lycopersicum* (tomato)	Tomato seedlings were flooded in phage suspension prior to flooding in bacterial suspension	Phage densities were quantified using droplet digital polymerase chain reaction	Hernandez et al. 2020
Pectobacterium atrosepticum	*Solanum tuberosum* (potato)	Tubers with previously made wounds were washed in distilled water supplemented with the phage cocktail, then inoculated with bacteria through the wounds	The amount of macerated tissue was calculated by subtracting the weight of the potato after the removal of macerated tissue from its original weight	Carstens et al. 2019
Pseudomonas syringae pv. *actinidiae*	*Actinidia deliciosa* (kiwifruit)	Leaves were treated with phage suspension on both sides using the silicon brusher and after 2 h of incubation plants were treated with bacteria the same way	Visible symptomatic spots frequency and sizes	Song et al. 2021

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Table 1 to be continued

Pathogen	Crop	Phage application procedure	Efficiency evaluation	Reference
Ralstonia solanacearum	*Solanum lycopersicum* (tomato) and *Solanum tuberosum* (potato)	Tomatoes were inoculated through roots (Singh et al. 2017), phages suspensions were poured around tomato seedlings in the soil. For potatoes, tuber slices were inoculated as described by (Czajkowski et al. 2015)	Disease symptoms of tomato plants were assessed using wilting grade scale	Umrao et al. 2021
Burkholderia glumae	*Oryza sativa* (rice)	Both healthy and *B. glumae*-infected seeds were soaked in the phage suspension and then sown in soil	Rot disease severity score	Sasaki et al. 2021
Pectobacterium odoriferum	*Brassica rapa* (Kimchi cabbage)	Both bacteriophages and pathogens were sprayed on seedlings	Lesion lengths and visual symptoms	Lee et al. 2021
Pectobacterium atrosepticum, P. carotovorum subsp. *carotovorum*	*Solanum tuberosum* (potato)	Halved-tubers of potato were soaked in phage/bacteria suspension	Area of macerated tissue	Zaczek-Moczydłowska et al. 2020
Cronobacter spp.	*Lactuca sativa* (lettuce)	Leaves previously treated with bacteria were covered with phage suspension	Viable bacterial counts, colony forming units/mL	Luo et al. 2021

that have been experimentally verified *in vitro* can explain the patterns observed in natural communities. Hernandez and Koskella (2019) used lytic phages and their bacterial host *P. syringae* to provide a direct comparison of phage resistance evolution between two distinct environments: a high-nutrient medium (*in vitro*) and a tomato plant (*in planta*). The authors provided evidence of the rapid and frequent phage resistance evolution *in vitro*, but not *in planta*, explaining it through environmental pressure and the high costs of resistance for the bacteria. On the other hand, the lower frequency of phage resistance *in planta* could be explained by phage decaying over time, the lack of phage replication or phage-resistant cells dying and, thus, the phage becoming inactive. This work also suggests that the effectiveness of agricultural phage therapy may be limited more by low phage replication rather than high rates of resistance evolution in bacteria. Similarly, studies of medical bacteriophages report the frequency of resistance developed *in vivo* during phage therapy being substantially lower in comparison to the resistance rates observed *in vitro* (Kutter et al. 2010).

Challenges to the widespread use of phage therapy. Despite all the advantages summarised above, we are still far from the large-scale implementation of phages in plant pathogen controls. There are still several challenges to overcome until phages can be widely used to control pathogenic bacteria. First, the high specificity of the phages, limiting the spectrum of the target bacteria; the solution to this problem could be the use of polyvalent phages or phage cocktails. Second, the emerging phage resistance in bacteria, that develops very fast and can be transmitted horizontally. However, this is not a big issue for phage therapy; as the phage resistance often causes a loss in the bacterial fitness, speed of growth or virulence. Next, the self-elimination of phages from the environment that might result in the need to repeat the treatment and to develop protective formulations. Finally, phage sensitivity to the environmental factors, such as UV radiation or desiccation; this can be partially solved by applying phage suspensions at the end of the light photoperiod and/or adding protective components into the mixture.

An additional concern of phage field application is a lack of knowledge about possible interactions between phages and plants. It is generally accepted that phages do not interact with plants directly, so

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the effects of phages on plant hosts are rather overlooked. However, a number of phage-like genes have been identified in plants (Buttimer et al. 2017), likely as a result of the horizontal transmission by bacteria. A common way to present a successful trial of phage preparations is by showing a picture of a plant with disease symptoms against an asymptomatic one. However, the effect of phages *per se* on plant metabolism is poorly investigated, as well as the signalling cross-talk initiated by the recognition of products of the phage action, i.e., mixture of phage particles with the debris of bacterial cells after lysis. Indeed, some phage products acted more efficiently together with systemic acquired resistance inducers (Obradovic et al. 2004; Lang et al. 2007; Ibrahim et al. 2017), decreasing the hypersensitive response while keeping the antibacterial efficiency (Obradovic et al. 2005). For instance, acibenzolar-*S*-methyl treated plants became necrotic within 48 h after inoculation with bacteria, showing a hypersensitive type of reaction. On the contrary, plants treated with a combination of acibenzolar-*S*-methyl and phages showed almost no symptoms, as the phage application decreased the bacterial population on the leaf surface, thus reducing the ingress and intensity of the plant response. A significant reduction in the intensity of the plant response was also achieved when acibenzolar-*S*-methyl, isolated with a bacterial hairpin protein and phages were applied to the same leaf (Obradovic et al. 2005). Next, it remains unclear whether phages are able to penetrate plant tissues (through natural openings, like the stomata, or mechanical wounds), and if so – whether phages remain virulent inside plants and for how long. Phages were found in the upper tissues of tomato plants up to 15 days after the soil application, depending on the roots' health status condition (as root damage reduced the phage adsorption capacity) (Iriarte et al. 2012). However, phage persistence was consistently higher on leaflets from plants co-treated with phages and attenuated bacterial strains compared with leaflets that only received the phage. It is thus suggested that the application of attenuated bacteria strains can prolong the phage persistence inside plants. In the experiments of Kolozsváriné Nagy et al. (2015), *Erwinia amylovora*-specific bacteriophages were capable of translocation into tissues of apple seedlings, and were detectable by real-time qPCR in the aboveground plant parts after application to the roots. Vice versa, phages could be detected in roots after spraying them on the leaves and stem. Interestingly, the pre-treatment of plants with phages and the following exposure to natural bacterial communities decreased the accumulation of amino acids and nitrogen-containing compounds, while increasing the accumulation of citrate (Papaianni et al. 2020). The authors suggested that the presence of the phage stimulated the conversion of the amino acid carbon skeleton into precursors/intermediates of the Krebs cycle, in order to support the mitochondrial metabolism and the production of adenosine triphosphate. In conclusion, the mode of action of different phage species is quite variable; also, phages may behave differently under *in vivo* and *in vitro* conditions. More research is, thus, needed to decipher the phage action *in vivo*, especially in phage-bacteria-plant communication, as each participant can determine the outcome of the interaction.

CONCLUSION

Despite the active research towards the use of phage products against plant bacterioses, the practical application of such knowledge is still limited. One of the reasons for such a limitation is the heterogeneity of the research strategies and the absence of commonly accepted protocols to evaluate the phage efficiency in plants. Furthermore, a deeper investigation of the effect of phage suspensions and products of their interaction with bacteria on plants, especially on the plant innate immune system, is needed to open new directions in the treatment and prevention of plant diseases.

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