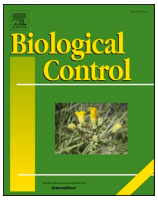
Biological Control 189 (2024) 105444

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon



Lytic phages isolated from Egypt for biocontrol of potato soft rot caused by *Pectobacterium carotovorum*

Kamel M. Elhalag [a,1], Mohamed A. Nasr-Eldin [b,1], Qi Huang [c,*], Abd-El-Aziz M. Rabab [a], Abdelmonim Ali Ahmad [d,*]

[a] *Bacterial Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt*
[b] *Botany and Microbiology Department, Faculty of Science, Benha University, Benha 13511, Egypt*
[c] *Floral and Nursery Plants Research Unit, United States National Arboretum, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, USA*
[d] *Department of Plant Pathology, Faculty of Agriculture, Minia University, El-minia 61519, Egypt*

HIGHLIGHTS

- Two novel phages, PcaP1EGY and PcaP2EGY, were isolated from soil in Egypt.
- The phages are specific to soft-rot-causing pathogen *Pectobacterium carotovorum*.
- The two lytic podoviruses are characterized and their genome sequences determined.
- The phages exhibited remarkable survivability under a variety of tested conditions.
- The two phages offered significant protection against soft rot disease in potatoes.

ARTICLE INFO

Keywords:
Pectobacterium carotovorum
Bacterial soft rot
Lytic phage
Stability
Biocontrol

ABSTRACT

Pectobacterium carotovorum is an economically important phytopathogen causing destructive bacterial soft rot disease in many ornamental plants and fruit and vegetable crops worldwide, including potato. Phage therapy is a promising and environmentally safe alternative to combat bacterial diseases either in the field or during storage. In this study, we characterized two novel *P. carotovorum*-specific phages, designated PcaP1EGY and PcaP2EGY, isolated from soil in Egypt. We also sequenced the genomes of the two phages, which are the first two complete genome sequences determined for *P. carotovorum*-infecting podoviruses from Egypt. Phages PcaP1EGY and PcaP2EGY have a high phage titer of 10^{12} PFU/mL and growth characteristics of an exclusively lytic life cycle. Both phages displayed specificity to and visible lysis activity against all four tested strains of *P. carotovorum*. They both had a latent period of 30 min and a high burst size of approximately 599 PFU/infected cell for PcaP1EGY and 570 for PcaP2EGY. Additionally, they exhibited remarkable survivability under a wide range of pH, temperature, salt concentration and UV exposure conditions. Both phages, when used alone or in combination, significantly reduced *in vitro* growth of *P. carotovorum* after 4 h at all tested multiplicity of infection. Phages PcaP1EGY and PcaP2EGY offered significant protection for both potato tubers and potato plants against soft rot disease caused by *P. carotovorum* in both our tuber maceration test and greenhouse pot experiment as compared to tubers/plants inoculated with *P. carotovorum* without receiving any phage treatment. Our results suggest that phages PcaP1EGY and PcaP2EGY have great biocontrol potential against potato soft rot disease caused by *P. carotovorum* either in the field or during storage after harvest.

1. Introduction

Potato (*Solanum tuberosum L.*) is considered the most important vegetable crop followed by tomato (FAOSTAT, 2022). It is an economically attractive crop in both the agricultural production sector as well as exportation processes to various countries worldwide (Haverkort and

* Corresponding authors.
E-mail addresses: qi.huang@usda.gov (Q. Huang), abdelmonim.ali@mu.edu.eg (A. Ali Ahmad).
[1] **These authors contributed equally to this work and share first authorship.**

https://doi.org/10.1016/j.biocontrol.2024.105444
Received 18 November 2023; Received in revised form 15 January 2024; Accepted 20 January 2024
Available online 24 January 2024

Struik, 2015). Annual production of potato was ranked first among root and tuber crops with a production rate of 330 million metric tons from an area coverage of 18,651,838-hectare (Tolessa, 2018). Egypt is considered one of the largest exporters and producers of potatoes in Africa with a total annual potato production of 5 million tons per year (Rabia et al., 2021). One limitation for potato production and exportation is the associated diseases that negatively affect potato quality and quantity. One of these diseases is bacterial soft rot that is responsible for more yield losses than any other bacterial diseases. Post-harvest yield losses in potato due to bacterial soft rot have been estimated to vary between 15 and 30 % (Abo-Elyousr et al., 2010).

Bacterial soft rot diseases are severe problems for vegetable and fruit crops all over the world, causing crop losses up to 40 % (Mantsebo et al., 2014) with varying degrees of disease severity depending on weather, plant susceptibility, and pathogen inoculum. Among the most economically important hosts affected by bacterial soft rot worldwide are potato, carrot, tomato, onion, maize, and rice (Bhat et al., 2010; Charkowski, 2018). Bacterial soft rot is caused by members of the plant pathogenic soft rot *Pectobacteriaceae* (SRP) containing Gram-negative pectolytic bacteria that are ubiquitously present on the surface of potato seed pieces and in soil. *P. carotovorum* subsp. *carotovorum*, *P. aroidearium*, *P. atrosepticum*, *P. brasiliense*, *P. betavasculorum* and *P. wasabiae* are all members of SRP and were identified to cause soft rot both in storage and in transit (Czajkowski et al., 2015; Nabhan et al., 2013; Pérombelon, 2002; Gardan et al., 2003; Adeolu et al., 2016; Charkowski, 2018; Portier et al., 2019; Agyemang et al., 2020). Recent reclassification has placed the following 20 species in the genus *Pectobacterium*: *P. aquaticum, P. aroidearum*, *P. atrosepticum*, *P. brasiliense*, *P. cacticidum, P. betavasculorum*, *P. carotovorum*, *P. actinidiae*, *P. odoriferum*, *P. peruviense*, *P. quasiaquaticum*, *P. polaris*, *P. parmentieri*, *P. parvum*, *P. fontis*, *P. punjabense*, *P. versatile*, *P. polonicum*, *P. wasabiae* and *P. zantedeschiae* (Pritchard et al., 2016; Zhang et al., 2016; Portier et al., 2019; Toth et al., 2021).

P. carotovorum affects a wide range of crops including potato and causes soft rot disease that can be developed during plant storage, transport or in the field, leading to crop losses, food shortages and significant economic losses globally (Charkowski, 2018; Jee et al., 2020). *P. carotovorum* enters a susceptible host through wounds or natural openings such as stomata and lenticels and causes soft rot of plant tissues by producing plant cell wall degrading enzymes such as pectate lyase, galacturonase and cellulase (Toth et al., 2003; Narváez-Barragán et al., 2020). The bacterium is transmitted by contaminated water and infested soil, as well as latently infected plant materials for long distant spread. In Egypt, *P. carotovorum* causes soft rot and blackening of potato tubers in fields, and during transit and storage under warm and temperate conditions (Rashid et al., 2012).

Every year, the severity and occurrence of soft rot increases greatly, and the disease causes significant losses in potato crop (Toth et al., 2011). The disease has become one of the major problems of potato in the world, but currently, no effective control methods are available to combat the disease. Although antimicrobial pesticides have been utilized to prevent crop and plant diseases, they pollute environment and increase pesticide resistance of their target microbes (Garvey, 2022), and the large-scale use of antibiotics is no longer effective against phytopathogens (Aslam et al., 2018). The capability of bacterial pathogens to develop resistance to chemicals and the concern about the negative impact of chemicals on human health have necessitated exploration of environmentally safe alternative bactericides.

Bacteriophages or phages have the potential to be a viable and eco-friendly alternative to antimicrobial pesticides in agriculture. Phages are viruses that infect and replicate in bacteria. When environment is favorable to infection, lytic phages reproduce quickly to efficiently reduce bacterial populations without negatively affect non-targeted beneficial microflora. Phages have been applied successfully to protect potatoes against SRP (Adriaenssens et al., 2012; Carstens et al., 2019; Voronina et al., 2019; Beňo et al., 2022).

The first sequenced *P. carotovorum* subsp. *carotovorum*-infecting *Podoviridae* phage PP1 was reported by Korean scientist in 2012 (Lee J. et al., 2012). The phage has a genome of 44,400 bp in size and a GC content of 49.74 % with 48 ORFs but no tRNA (Lee J. et al., 2012). The *Siphoviridae* phage My1 is another *P. carotovorum*-infecting phage sequenced by Korean scientists in the same year (Lee D. et al., 2012). In addition, additional 12 novel phages within the order Caudovirales were isolated and reported (Lim et al., 2013; Lim et al., 2014; Kalischuk et al., 2015; Lim et al., 2015; Lim et al., 2017; Buttimer et al., 2018a; Buttimer et al., 2018b; Voronina et al., 2019; Buttimer et al., 2020; Lukianova et al., 2020; Pedersen et al., 2020; Naligama and Halmillawewa, 2022). Studies by Czajkowski et al. (2015) and Smolarska et al. (2018) showed the potential of several well-studied soft rot pathogen-infecting phages as biocontrol because of their significant antibacterial activities against their bacterial hosts.

Most *Pectobacterium*-infecting phages belong to the family *Autographiviridae*, referred to as short-tailed phages resembling the model phage T7 (Evseev et al., 2020). Various environmental factors influence the success of phage-based biocontrol in agriculture, including levels of bacterial population, phage titers at the application site (Abedon et al., 2017), temperature, pH of the rhizosphere, moisture, organic content of the soil (Czajkowski et al., 2017), and the possible presence of phage–resistant bacterial mutants evolved probably due to modifications of phage receptors in bacteria or development of bacterial defense mechanisms against phages (Svircev et al., 2018). To counteract the occurrence of phage-resistant bacteria, formulations containing multiple phages have been tested recently against losses caused by SRP (Buttimer et al., 2018a; 2018b; Carstens et al., 2019).

Potato is an economically important crop worldwide, especially in Egypt. It is estimated that 22 % of potatoes are lost yearly due to diseases caused by plant pathogens and pests (Czajkowski et al., 2011). Soft rot, caused by bacterial pathogens including *P. carotovorum*, in potatoes alone is responsible for 30 to 50 % of the yield losses (Rahman et al., 2012). As a result, research is urgently needed to explore eco-friendly approaches such as the use of phages to control soft rot disease which would not only reduce the reliance on chemical pesticides but also avoid the potential development of pesticide resistance. This study is therefore undertaken to isolate *P. carotovorum*-infecting phages from potato-growing regions in Egypt, characterize the phages biologically and molecularly including determining their complete genome sequences, and explore their biocontrol potential against *P. carotovorum* by determining their effect on *in vitro* growth of *P. carotovorum* and suppression of soft rot disease on potato tubers and plants. To the best of our knowledge, the complete genome sequences of PcaP1EGY and PcaP2-EGY we reported here are the first two for lytic *P. carotovorum*-infecting phages isolated in Egypt.

2. Materials and methods

2.1. Strains and media used in this study

The virulent strain 100H of *P. carotovorum* was used for phage isolation and biological characterization, as well as inoculation of potato tubers and potato seedlings. The strain was previously isolated by Elhalag et al. (2020) from a pilot survey targeting potato plants/tubers showing typical symptoms of soft rot and blackleg diseases in different locations in Egypt. Other *Pectobacterium* and non-*Pectobacterium* strains listed in Table 1 were used to determine the host range of isolated phages. All pathogenic *Pectobacterium* and *Ralstonia* strains were regularly maintained at the culture collection of Bacterial Diseases Research Department, Agricultural Research Center, Giza, Egypt. Other pathogenic or antagonistic bacterial strains in Table 1 were kindly provided by the Bacterial Diseases Research Department. All bacterial strains used in this study were grown on nutrient agar or King's medium slants (King et al., 1954) for routine testing and maintenance of bacteria (short-term usage), while long-term preservation was done at −30 °C in a final

Table 1
Host range of phages PcaP1EGY and PcaP2EGY.

Bacterium		Strain[a]	Spot test*		Plaque assay*	
			PcaP1EGY	PcaP2EGY	PcaP1EGY	PcaP2EGY
Pathogenic	*Pectobacterium carotovorum*	100H	+	+	+	+
		GH1	+	+	+	+
		GH2	+	+	+	+
		GH5	+	+	+	+
	P. atrosepticum	MH3c	–	–	–	–
		Fel2	–	–	–	–
		MH1	–	–	–	–
		MH2	–	–	–	–
		MH6	–	–	–	–
		MH8	–	–	–	–
		MH10	–	–	–	–
		MH11	–	–	–	–
	P. aroidearum	P.ash	–	–	–	–
	Ralstonia solanacearum (Phylotype IIA sequevar 1)	K3	–	–	–	–
		*K*10	–	–	–	–
		K16	–	–	–	–
	Erwinia amylovora	Ea	–	–	–	–
	Rhizobium radiobacter (*Agrobacterium tumefaciens*)	AGt	–	–	–	–
	Robbsia andropogonis (*Burkholderia andropogonis*)	Ba	–	–	–	–
Antagonistic	*Serratia marcescens*	100B	–	–	–	–
	Pseudomonas fluorescense	200B	–	–	–	–
	Stenotrophomonas maltophilia	300B	–	–	–	–
	Bacillus thuringiensis	400B	–	–	–	–
	B. subtilis	500B	–	–	–	–
	P. putida	600B	–	–	–	–
	P. aeruginosa	177	–	–	–	–
	Enterobacter aerogenes	114	–	–	–	–
	P.japonica	447	–	–	–	–

[a]All *P. carotovorum*, *P. atrosepticum* and *R. solanacearum* strains used in this study were previously isolated and identified by Elhalag et al., 2015, 2020). Other bacterial strains were kindly provided by Bacterial Diseases Research Department, Plant Pathology Research Institute, Giza, Egypt.
*"+" means that no plaques were observed, indicating the bacteria are not hosts of the phages, while "-" means clear plaques were observed, indicating the bacteria are hosts of the phages.

concentration of 20 % sterilized phosphate-glycerol solution (Hayward, 2000).

2.2. Phage isolation

Phages PcaP1EGY and PcaP2EGY were isolated using an enrichment method (Hammad et al., 2016). Clay soil samples were collected from potato-cultivating fields in Kafr-Shukr town, Al Qalyubia Governorate, Egypt. For phage isolation, a soil sample (10 g) was mixed with 100 mL of King's liquid media. Then 1 mL of the overnight culture of *P. carotovorum* strain 100H was added and incubated overnight at 28°C with continuous shaking. The culture was centrifuged at 6,000 rpm and the supernatant was filtered through 0.45 μm pore-size filter (PES filter with the catalogue number of SFM25PE0045S by cobetter®, Japan) to yield a bacterial cell-free phage lysate. One hundred microliters of the phage lysate were used as aliquots for plaque forming assay using the *P. carotovorum* strain 100H as the host bacterium. The plaque assay was carried out according to Mihara et al. (2016) using King's medium plates containing 2 % agar overlaid with 0.8 % King's soft agar. The plates were incubated at 28 °C for 24 or 48 h. The shape, size and number of plaques were examined visually by eyes and recorded after the incubation time. Spot test was also conducted to confirm plaque assay result as follows: *P. carotovourum* strain 100H bacterial cells (10^8 CFU /mL) were mixed with melted 0.8 % agar (50 °C) and the mixture was poured on top of a King's medium plate containing 2 % agar to make a double-layered agar plate. After the top layer solidified, 5 μL of the phage lysate was spotted on the double-layered plate. Appearance of bacterial lysis zones on the plate indicated the presence of phages.

2.3. Phage purification

Phage purification was done using the double-layered plaque assay (Kropinski et al., 2009). A single plaque was picked using a sterile pipette tip and the phage was suspended in 500 μL of the saline-magnesium (SM) buffer (50 mM Tris–HCl at pH 7.5, 100 mM NaCl, 10 mM MgSO4, and 0.01 % gelatine) (Lai et al., 2011). A pure phage isolate was obtained using the triple phage purification process (Addy et al., 2019). The plaque-forming unit (PFU/mL) was calculated based on the number of clear plaques and the dilution factor of the phages used.

2.4. Phage propagation

Phages were propagated by first growing *P. carotovorum* strain 100H in King's liquid medium with shaking for 16–22 h at 28 °C, then the concentration of the culture was adjusted to 10^8 CFU/mL before adding a purified phage isolate at a multiplicity of infection (MOI) of 1.0. After growing overnight at 28 °C, the mixture was centrifuged at 5,000 rpm for 5 min and the supernatant was filtered through a 0.45 μm filter to obtain a bacterial cell-free phage lysate. The phage titer was determined by plaque assay using serially diluted phage lysate. The propagated phage suspension was stored at 4 °C for further study.

2.5. Determination of phages' optimal MOI

The optimal MOI was determined for the two purified phages PcaP1EGY and PcaP2EGY. *P. carotovorum* strain 100H was infected with each phage at different MOIs (0.01, 0.1, 1.0, and 10). After overnight incubation at 28 °C, the mixture was centrifuged at 5,000 rpm for 10 min. The supernatant was filtered through a 0.45 μm filter and subjected to the plaque assay to determine the phage titer. The MOI with the highest phage titer (PFU/mL) was considered the optimal one.

2.6. Phage morphology

To determine the morphology of the purified phages PcaP1EGY and PcaP2EGY, 5 μL of the filtered phage lysates (approximately 10^{12} PFU mL^{-1}) were dropped onto carbon-coated copper grids and incubated at room temperature for 2 min, and the residual liquid was absorbed by filter paper. Then, the phage samples on the grids were negatively stained with 2 % aqueous uranyl acetate (UrAc, pH 4.0) for 2 min, and the extra UrAc solution was blotted off. The phages were observed under transmission electron microscope (TEM) (JEOL JEM-2100, Japan Electron Optics Laboratory Co., Ltd) at the Nano Tech Company, Egypt. Classification of the two phages was determined based on the guidelines of the International Committee on Taxonomy of Viruses (King et al., 2011).

2.7. One-step growth curve assay

One-step growth curve experiment was performed to determine the latent period and burst size of phages PcaP1EGY and PcaP2EGY as previously described by Adams (1959), with some modifications. Briefly, 5 mL of exponential-phase culture of *P. carotovorum* strain 100H was centrifuged at 5,000 rpm for 5 min at 4°C. The pellet was resuspended in 10 mL of King's liquid medium to obtain an OD of approximately 0.4 at 600 nm wavelength. The bacterial concentration was adjusted to 1 × 10^8 CFU/mL using sterile King's liquid medium. Each phage (10^8 PFU/mL) was added to its respective host bacterial suspension to achieve a MOI of 1.0. The mixture was left at room temperature for 10 min to allow the phage to be adsorbed to the surface of the host bacterial cells and then centrifuged. The pellet was resuspended in 10 mL of King's liquid medium and incubated at 28 °C with shaking (150 rpm) for 1.5 h. One hundred microliters were taken from each sample at 10-min intervals for 90 min and used for plaque assays with three replicates in each assay to determine the latent period, burst time, and phage relative burst size per infected cell according to El-Dougdoug et al. (2019).

2.8. Determination of phage host specificity

A host specificity assay was performed as described by Czajkowski et al. (2014) using a panel of four strains of *P. carotovorum*, eight strains of *P. atrosepticum,* and three strains of *Ralstonia solanacearum* (Table 1), which were previously isolated from symptomatic potato tubers and identified by Elhalag et al. (2015, 2020). In addition, 13 other plant pathogenic or antagonistic bacterial strains were also used. The 13 strains were originally isolated from soil by Farag et al. (2017) and kindly provided by the culture collection of Bacterial Diseases Department, Plant Pathology Research Institute, Giza, Egypt (Table 1). Phage host range was examined using both plaque assay and spot test according to Armon and Kott (1993). Non-phage inoculated plates were used as a negative control. The appearance of clear zones where the phage was applied to was scored as positive, whereas the absence of clear zones as negative. The plates were kept up to 48 h after phage inoculation for presence or absence of lysis zones. The experiment was repeated twice.

2.9. Phage stability under different temperature and pH conditions

The ability of phages PcaP1EGY and PcaP2EGY to withstand various environmental conditions was assessed in this study, in order to determine their potential as biocontrol agents. The viability of PcaP1EGY and PcaP2EGY was determined after incubating each of them at different temperatures and pH conditions. The pH of the SM buffer solution was adjusted using 1 M HCI or 1 M NaOH to obtain a pH range from 2 to 12. Phage aliquots (4.9 x 10^9 and 3.7 x 10^9 PFU/mL for PcaP1EGY and PcaP2EGY respectively) were added to the incubation buffer with a different pH and incubated for 1 h. After the incubation, phages were enumerated using the plaque assay with *P. carotovorum* strain 100H as the host bacterium. Thermal stability of the two phages was determined at 4, 28, 40, 50, 60, 70 and 80 °C, respectively. Phage aliquots in SM buffer were incubated for 1 h under each temperature condition and subjected to the plaque assay to estimate phage numbers (Czajkowski et al., 2014). There were three replicates for each time point, and the experiment was repeated three times.

2.10. Phage stability under different salt concentration and UV radiation conditions

To study the effect of salt concentrations on phage stability, phages PcaP1EGY and PcaP2EGY were tested under different NaCl concentrations ranging from 2 to 10 %. To do that, phage aliquots were added to different concentrations of NaCl solution with a pH of 7 and incubated for 1 h at room temperature. After the incubation, phages were enumerated using the plaque assay with *P. carotovorum* strain 100H as the host bacterium.

Phage stability under UV radiation was tested according to Ramirez et al. (2018) with simple modifications. Briefly, 1 mL of each phage lysate (3x 10^{12} and 1.23 x 10^{12} PFU/mL for PcaP1EGY and PcaP2EGY, respectively) was irradiated for 10, 20, and 30 min under the UV light (λ = 365 nm; 320 mW/m2), respectively. The number of the phages survived after each radiation was determined by the plaque assay.

For both the salt concentration and UV irradiation experiments, three replicates were used in each tested concentration or radiation duration, and each experiment was repeated three times.

2.11. Phage DNA extraction, genome sequencing, and genomic analysis

Phage DNA was extracted from a purified phage particle suspension with at least 10^8 PFU/mL using the Phage DNA Isolation kit (Norgen Biotek Corp, Canada) following the manufacturer's instructions. Extracted DNA was stored in a 1.5 mL microcentrifuge tube at − 20 °C until use. It was subjected to enzymatic digestions using standard molecular biology methods. Phage DNA was sequenced commercially on an Illumina MiSeq with 2 × 150 bp reads and the genome sequence assembled using Spades v3.11 by SeqMatic (Fremont, California). Potential open reading frames (ORFs) larger than 30 amino acids (aa) were predicated using the software described by Besemer (2001). Homology searches to assign possible functions for each predicted ORF were performed using BLASTN and BLASTP (Altschul, 1997), respectively. An e-value threshold of e − 4 or less was used for two proteins to be considered a match.

Complete genome sequences of phages PcaP1EGY and PcaP2EGY were blasted in GenBank by BLASTn to find their closely related phages. For phylogenetic analysis, aa sequences of the phage major capsid protein, DNA polymerase, RNA polymerase and terminase were first aligned using MUSCLE (MUltiple Sequence Comparison by Log-Expectation) (https://www.ebi.ac.uk/Tools/msa/muscle/). A phylogenetic tree was then constructed using the software Geneious prime 2022 (https://www.geneious.com).

2.12. Phage effect on in vitro growth of P. carotovorum

King's broth medium was inoculated with an overnight culture of *P. carotovorum* strain 100H grown at 28 °C with shaking. When the bacterial culture grew to the early exponential phase (10^8 CFU/mL), each of the phages PcaP1EGY, PcaP2EGY and their combination (cocktail) [PcaP1EGY: PcaP2EGY = 1:1 (v/v)] was added at a MOI of 1, 10, and 100 respectively. Bacterial growth measured by OD_{600} nm was done every 2 h for 12 h using SPECTROPHOTOMETER 2000 (UNICO, HongKong). As a negative control, bacterial cultures were inoculated with the same volume of SM buffer instead of phages. The experiments were performed independently three times with three replicates per treatment in each experiment.

2.13. Bioassay using potato tubers

Potato tubers (cv. Cara or Spunta) were surface sterilized by soaking in 70 % ethanol for 10 min, washed with sterilized distilled water, and air dried under the laminar flow hood. One well (5-mm diameter) was made in each tuber using a sterile corkborer. One hundred microliters of *P. carotovorum* (2 x 10^8 CFU/mL) was added to the well, before each of the two phages PcaP1EGY and PcaP2EGY or their cocktail was added at a MOI of 10^3 and 10^4, respectively. Four potato tubers were used for each treatment. Inoculation with sterile SM buffer was used as a negative and *P. carotovorum* alone as a positive control. Four tubers inoculated with phage PcaP1EGY and the other four with phage PcaP2EGY were used as two phage-only control treatments. The diameter of the rotten tissues around the wells was measured after incubation at 28 °C for 72 h. The experiment was replicated twice, and the diameters were averaged for each treatment.

2.14. Phage effect on suppression of disease caused by P. carotovorum in potato plants growing from tubers in pots

Potato tubers (cv. Spunta) were used to assess the effect of the two lytic phages PcaP1EGY and PcaP2EGY on disease caused by *P. carotovorum* in potato plants growing in pots. Plastic pots (30 cm in diameter) were sterilized by spraying with 70 % ethanol, then filled with 5 kg of autoclaved clay soil that is free from pathogenic bacteria. The clay soil has a pH of 7.6 and contains 77.18 % clay, 5.0 % silt, and 17.82 % sand. One potato tuber was planted in each pot at a depth of 7 cm from the soil surface. All the pots with potato tubers were regularly watered. Fourteen days after planting when two to three potato seedlings appeared, potato roots were gently wounded using a sterilized syringe to create wounds to increase the chance of the bacterium to enter the potato plants. Fifty milliliters of a 24-h-old culture of *P. carotovorum* strain 100H (2 x 10^8 CFU/mL) was then poured into each pot. To prepare the phage treatment, each phage (10^{12} PFU/ mL) was diluted with sterilized tap water for a final concentration of 7.2 x 10^{11} PFU/mL and used as a stock. Fifty milliliters of each phage stock were applied twice to the bacterial inoculated potato plants, once as a soil drench right after bacterial inoculation and the second time as a foliar spray five days after the first phage application. There were six treatments in the experiment: 1) water control – plants were inoculated and treated with water, 2) pathogen (Pc) control - plants were inoculated with *P. carotovorum* and treated with water, 3) Pc + PcaP1EGY - plants were inoculated with *P. carotovorum* and treated with phage PcaP1EGY, 4) Pc + PcaP2EGY - plants were inoculated with *P. carotovorum* and treated with phage PcaP2EGY, 5) phage PcaP1EGY control - plants were inoculated with water and treated with phage PcaP1EGY, and 6) phage PcaP2EGY control - plants were inoculated with *P. carotovorum* strain 100H and treated with phage PcaP2EGY. The two phage controls were included to assess if any physiological disorder might be resulted from the phage treatments. The plants were examined each day visually after seedling appearance (5–7 days after planting) to determine the number of wilted leaves and a total number of leaves per plant or per pot. Ten potato pots were used for each treatment, and the experiment was repeated two times. Disease incidence (DI) and treatment efficiency (TE) by phages were measured using the following formulas based on Kheirandish and Harighi (2015).

$DI = Numberofwiltedleavesperpot/Totalnumberofleavesperpot \times 100$

TE = (DI in pathogen control - DI in phage treatment)/DI in pathogen control × 100. The area under the disease progress curve (AUDPC) was calculated till the complete death of the pathogen control plants (10 days after soil drenching inoculation of *P. carotovorum*) according to Ilicic et al. (2023). Bacterial population in the soil rhizosphere were determined by dilution plating on Logan's medium (Fahy and Hayward, 1983) for each treatment 30 and 70 days after soil inoculation with *P. carovotorum*. The yield of potato tubers per pot was measured 100 days after soil inoculation and expressed as g/pot. For estimation of *P. carotovorum* population and measurement of potato tuber yield, the assessment trial was repeated twice.

2.15. Statistical analysis

Means ± standard deviation (SD) for all experiments in our study were calculated based on the number of replicates for each assessment as indicated in Material and Methods. Bars representing standard deviation were added to figures when appropriate.

2.16. Phage genome accession numbers

The complete genome sequences of *Pectobacterium* phages PcaP1EGY and PcaP2EGY were deposited into GenBank under the accession numbers OR599645 and OR599646.

3. Results

3.1. Isolation, morphology, and characterization of phages PcaP1EGY and PcaP2EGY

Two *P. carotovorum*-infecting phages were isolated from soil collected from the potato-cultivating fields of Kafr-Shukr town in Al Qalyubia Governorate, Egypt. Both phages produced clear circular lytic plaques (Supplementary Fig. 1). The average size of the plaques produced by PcaP1EGY is 2 to 3 mm (n = 15), while the ones by PcaP2EGY 3 to 4 mm (n = 15) in diameter (Supplementary Fig. 1). The phage titers of PcaP1EGY and PcaP2EGY were determined to be 3 × 10^{12} and 1.5 × 10^{12} PFU/mL, respectively.

Both PcaP1EGY and PcaP2EGY have icosahedral heads that are 72.40 ± 3.65 and 74.43 ± 2.33 (n = 5) nm in diameter, respectively (Fig. 1). They also have short non-contractile tails with a length of 14.30 ± 1.6 and 23.4 ± 0.61 nm (n = 5), respectively (Fig. 1). Since their morphology is typical of the podoviruses, they are named PcaP1EGY and PcaP2EGY by following the systematic phage naming approach proposed by Ahmad et al. (2017) because they are the first and second *P. carotovorum*-infecting phages belonging to the family *Podoviridae* that were isolated from Egypt.

3.2. Host range, optimum MOI, and infection cycle

Phages PcaP1EGY and PcaP2EGY infected and actively lysed all 4 strains of *P. carotovorum* used in this study (Table 1). They, however, did not infect any of the other 14 tested pathogenic or antagonistic bacterial species, including *P. atrosepticum, P. aroidearum, R. solanacearum, Erwinia amylovora, Rhizobium radiobacter* (formerly *Agrobacterium tumefaciens*), *Robbsia andropogonis* (formerly *Burkholderia andropogonis*), *Serratia marcescens, Pseudomonas fluorescence*, *P. putida*, *P. aeruginosa*, *Stenotrophomonas maltophilia*, *Bacillus thuringiensis*, *B. subtilis*, and *Enterobacter aerogenes* (Table 1), indicating their specificity to *P. carotovorum* (Table 1).

The optimal MOI of phages PcaP1EGY and PcaP2EGY was determined to be 1.0, based on the average count of PFU/mL of 4.8 x 10^{11} for PcaP1EGY and 2 x10^{11} for PcaP2EGY at that MOI from three separate experiments, which were the highest compared to the average count of PFU/mL at other tested MOIs of 0.1 (2x10^{10} for PcaP1EGY and 5.5x10^9 for PcaP2EGY), 10 (2.3x 10^{11} and 1.2x10^{11}) and 100 (1.1x 10^{11} and 5.7x 10^{10}).

The one-step growth curves of PcaP1EGY and PcaP2EGY propagated on *P. carotovorum* were determined (Fig. 2). Both phages had a lytic infection cycle of 50 min and a latent period of 30 min (Fig. 2). The burst size of PcaP1EGY was determined to be 599 PFU/infected cell, while that of PcaP2EGY was determined to be 570 PFU/infected cell (Fig. 2).

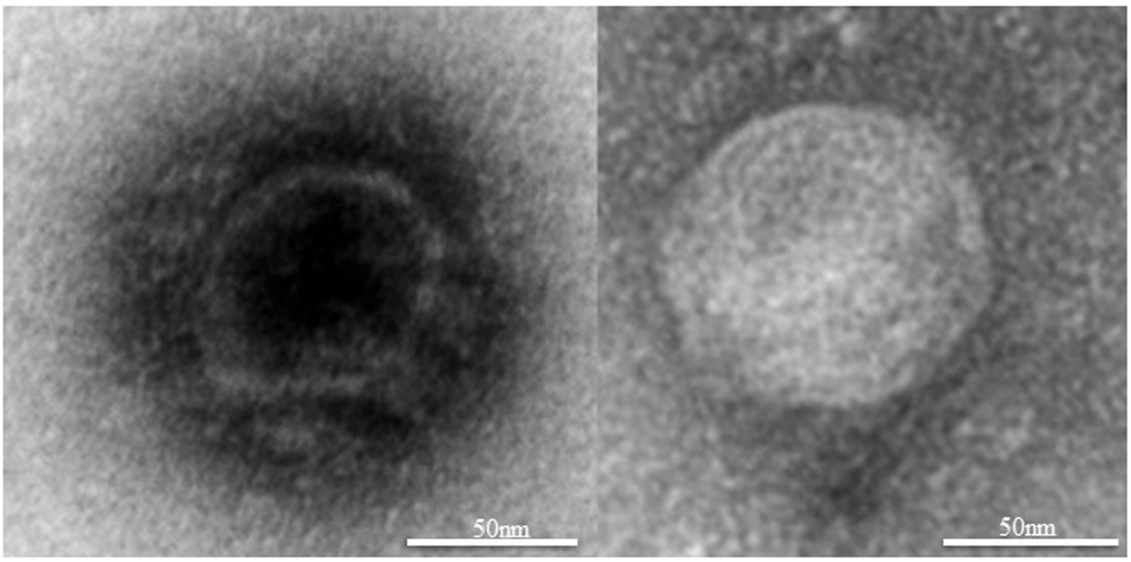


Fig. 1. Transmission electron micrograph of the purified phage virions of PcaP1EGY (left) and PcaP2EGY (right). The scale is indicated.

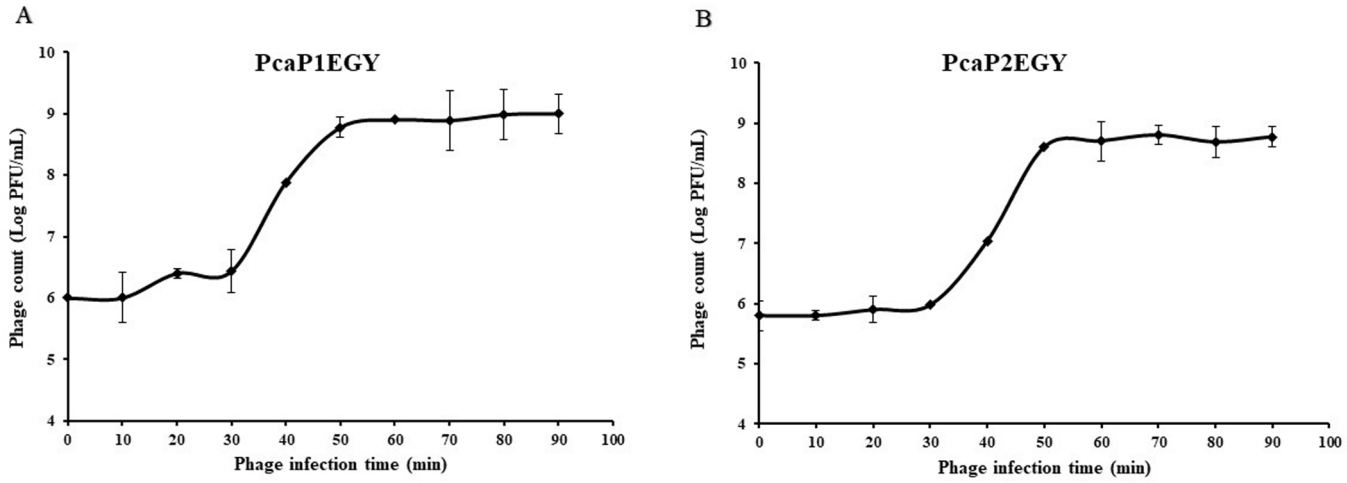


Fig. 2. One-step growth curve of phages PcaP1EGY (A) and PcaP2EGY (B) isolated from Egyptian soil against *P. carotovorum.* Means at each time points are based on two separate experiments, each containing three replicates. Bars represent standard deviation.

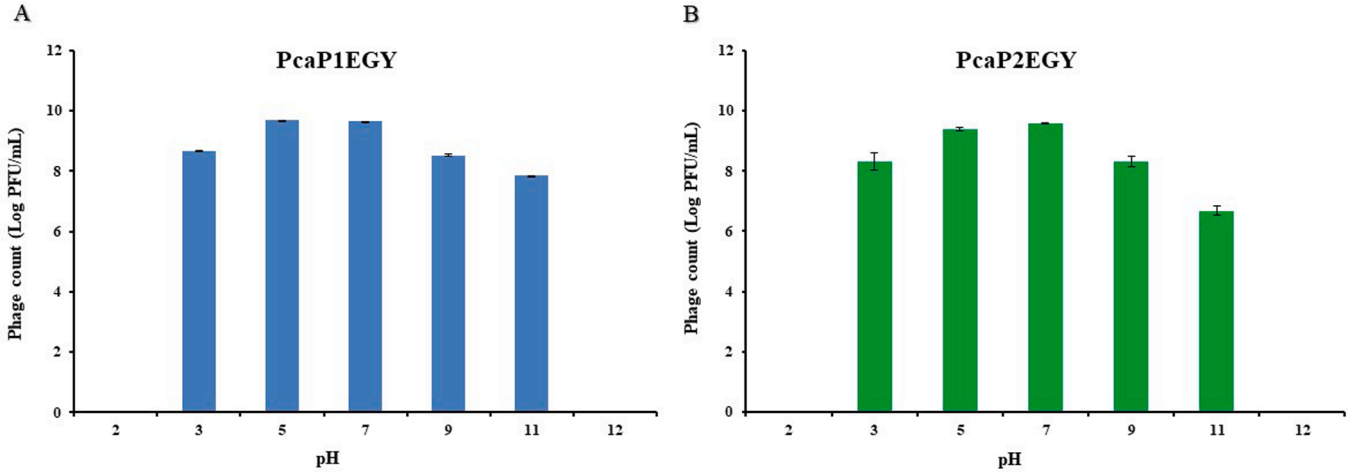


Fig. 3. Stability of phages PcaP1EGY (A) and PcaP2EGY (B) at different pH. PcaP1EGY ($4.9x10^9$ PFU/mL) and PcaP2EGY ($3.7x10^9$ PFU/mL) were incubated under each pH condition. The phage numbers were estimated by plaque assay using *P. carotovorum* as a host 1 h after incubation. All values represent means of three determinations. Bars represent standard deviation.

3.3. Stability of phages PcaP1EGY and PcaP2EGY at different pH, temperature, UV exposure and NaCl concentration conditions

Both phages survived at the pH range of 3 to 11, but the optimal pH for both phages was determined to be from 5 to 7, since their titers at that temperature range remained similar to their starting titers of 4.9 x 10^9 (PcaP1EGY) and 3.7 x 10^9 (PcaP2EGY) (Fig. 3). No phage particles were detected at pH 2 and 12, suggesting that they are lethal pHs for the phages (Fig. 3).

Phages PcaP1EGY and PcaP2EGY maintained their viability when incubated at 4, 28, 40, 50, 60 and 70 °C for a period of 1 h, although no phage particles were detected at 80 °C (Fig. 4). The optimal temperature range for both phages was determined to be from 4 to 40 °C, since no significant losses in phage titers were observed under that temperature range (Fig. 4).

As shown in Fig. 5, phages PcaP1EGY and PcaP2EGY did not lose their titers significantly after exposure to UV irradiation for 10 min since they maintained their starting titer of $3x10^{12}$ (PcaP1EGY) and $1.23x10^{12}$ PFU/mL (PcaP2EGY), but their titers were significantly reduced after UV exposure for 20- and 30-min (Fig. 5).

Phage PcaP1EGY was stable after incubation for 1 h at NaCl concentrations ranging from 2 to 6 %, but its titer was significantly reduced after incubation at the salt concentration of 8 and 10 % (Fig. 6A). Phage PcaP2EGY, however, was stable at the salt concentrations of 2 and 4 %, and significant reduction in phage titers was observed after the phage was incubated at the salt concentrations of 6, 8 and 10 % for 1 h (Fig. 6B).

3.4. Genomic features of phages PcaP1EGY and PcaP2EGY

The genomes of both PcaP1EGY and PcaP2EGY were completely degraded by DNase I, but not by RNase A, S1 nuclease, or Exonuclease I (data not shown). The genomes were also digested into multiple bands by endonuclease *Xho*I (data not shown). These results indicate that both phages have double-stranded DNA genomes. The complete nucleotide sequences of the *P. caratovorum*-infecting phages PcaP1EGY and PcaP2EGY were determined and deposited into GenBank under the accession numbers of OR599645and OR599646 respectively.

a. Phage PcaP1EGY genome:

The genome of phage PcaP1EGY was determined to be 40,853 bp in length with a G + C content of 50 %. When its whole genome was used as a query to search NCBI's nucleotide collection by BLASTn, the phage was found to be closely related to the Pectobacterium phage Q19 (accession number MK290739) with 99 % nucleotide identity over 96 % coverage, Pectobacterium phage Jarilo (accession number NC_047963.1) with 83 % nucleotide identity over 50 % coverage, and Pectobacterium phage vB_PcaP_P15_PC2B6 (accession number ON995367.1) with 79 % nucleotide identity over 27 % coverage.

Forty-nine potential ORFs with starting codon of ATG or GTG were identified within the genome of PcaP1EGY (Fig. 7 A, Supplementary Table S1). Information including their positions, best homologs and predicted functions are summarized in the Supplementary Table S1. Among the 49 ORFs, 34 had homologs in GenBank with predicted functions involved in phage structure, DNA packaging, replication, transcription and lysis. Thirteen were predicted hypothetical proteins similar to the ones in Pectobacterium phage Q19. Two ORFs, 27 and 42, had no homologs in the database (Supplementary Table S1). The similarities in genomic organization among Pectobacterium phages PcaP1-EGY and Q19, as well as T7 phage suggest that PcaP1EGY is a T7-like phage (Fig. 7 A). Similar to other podoviruses, three functional gene clusters (Kawasaki et al., 2009; Dunn et al., 1983; and Lavigne et al., 2003) were identified in the genome of PcaP1EGY: cluster I for early genes including protein kinase (ORF43), RNA polymerase (ORF 44), and DNA ligase (ORF 48); cluster II for metabolism genes including inhibitors of host RNA polymerase (ORF 5), single stranded DNA binding protein (ORF 6), endonuclease (ORF7), endolysin (ORF9), DNA primase (ORF 10), two DNA polymerase (ORF14 and 16), and exonuclease (ORF20); and cluster III for structural and assembly genes including seven for head and tail morphogenesis (ORFs 23–26, 28, 29, and 34), four internal virion proteins (ORFs 30–33), one small (ORF 36) and one large (ORF 39) terminate subunit proteins (Supplementary Table S1). ORFs predicted to function in host-cell lysis include holin (ORF 35), Rz lysis (ORF 38), and putative transmembrane (ORF 40) proteins (Supplementary Table S1). No ORF was predicted in the genome of PcaP1-EGY to function in phage integration (Supplementary Table S1), suggesting that like most podoviruses, phage PcaP1EGY is a lytic phage that lyses host bacterial cells without a lysogenic stage.

b. Phage PcaP2EGY genome:

The genome size of phage PcaP2EGY is 51,385 bp in length, with a G + C content of 42 %. Phage PcaP2EGY was found to be most closely related to the unclassified Bacillus phage 1_ICo-2020 (accession number MT700412.1) with 97.7 % nucleotide identity over 88 % coverage.

Eight-eight ORFs with starting codons ATG or GTG were predicted within the genome of PcaP2EGY (Supplementary Table S2). The 88

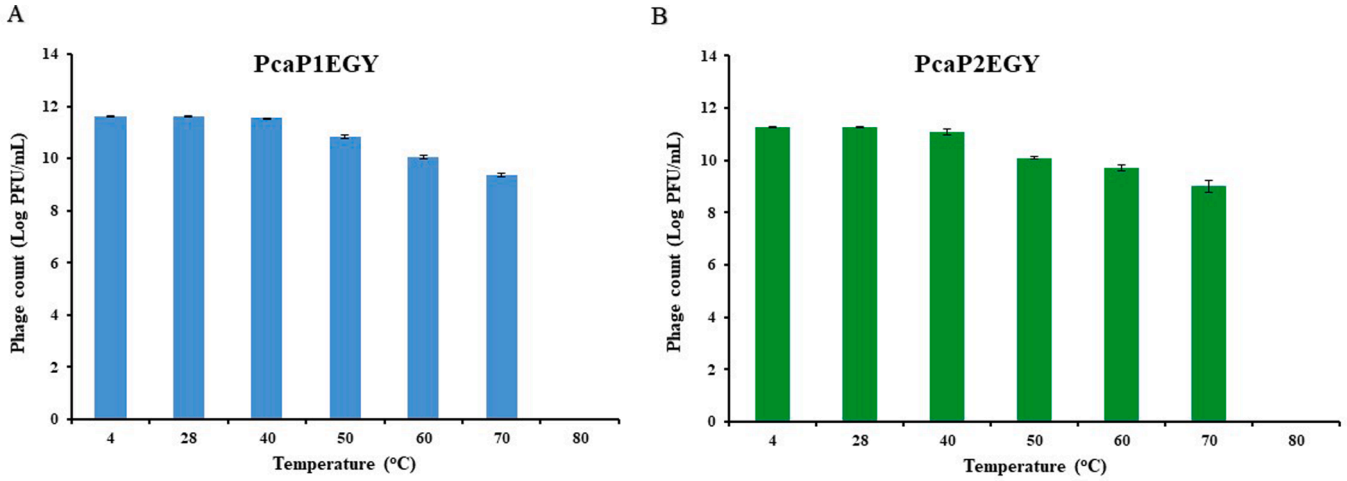


Fig. 4. Thermal stability of phages PcaP1EGY (A) and PcaP2EGY (B) at different temperature. PcaP1EGY ($4.13x10^{11}$ PFU/mL) and PcaP2EGY ($1.87x10^{11}$ PFU/mL) were incubated at each temperature point. The phage numbers were estimated by plaque assay using *P. carotovorum* as a host 1 h after incubation. All values represent means of three determinations. Bars represent standard deviation.

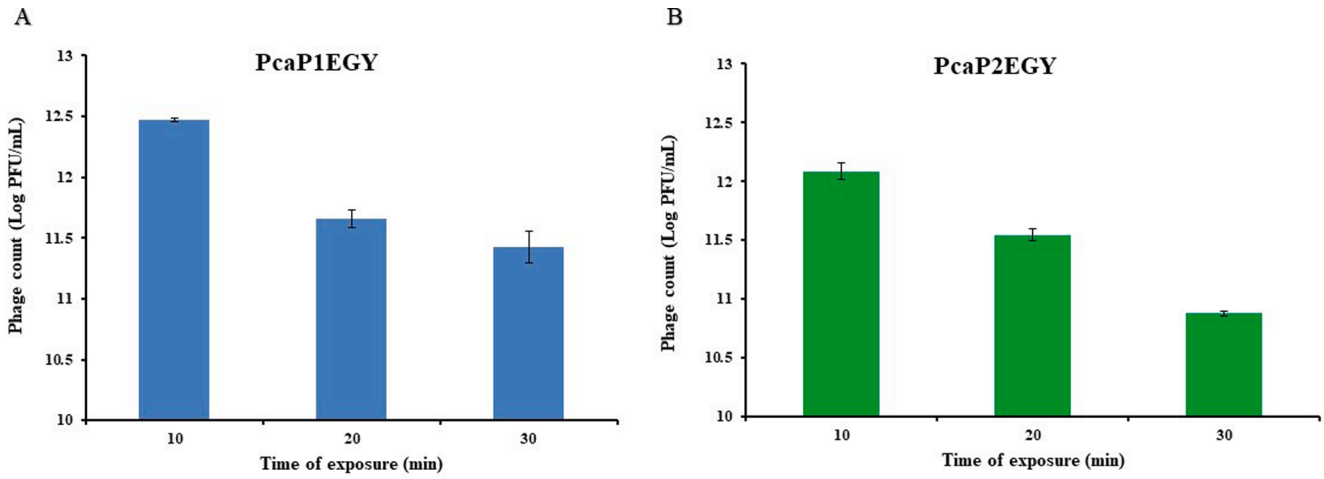


Fig. 5. Sensitivity of phages PcaP1EGY (A) and PcaP2EGY (B) to ultraviolet irradiation at 365 nm wavelength under different exposure time. PcaP1EGY ($3x10^{12}$ PFU/mL) and PcaP2EGY ($1.23x10^{12}$ PFU/mL) were exposed to the ultraviolet irradiation for indicated duration. The phage numbers were estimated by plaque assay using *P. carotovorum* as a host 1 h after incubation. All values represent means of three determinations. Bars represent standard deviation.

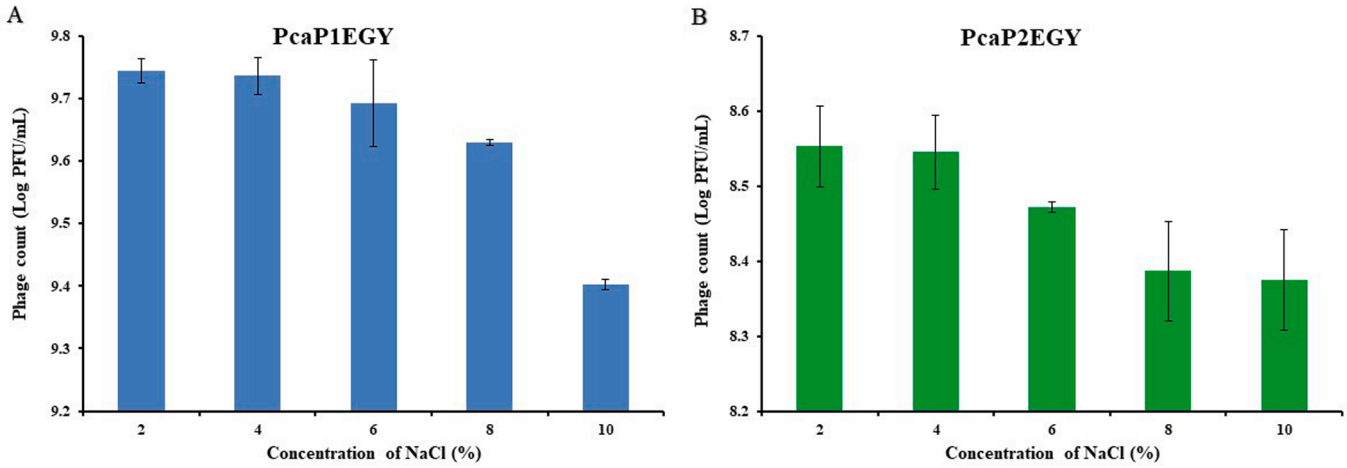


Fig. 6. Stability of phages PcaP1EGY (A) and PcaP2EGY (B) under different salt concentration. PcaP1EGY ($5.57x10^{9}$ PFU/mL) and PcaP2EGY ($3.9x10^{8}$ PFU/mL) were incubated under each indicated NaCl concentration. The phage numbers were estimated by plaque assay using *P. carotovorum* as a host 1 h after incubation. All values represent means of three determinations. Bars represent standard deviation.

ORFs were predicted to encode for (i) 32 proteins that had homologs in GenBank with known functions, including phage structural proteins, DNA packaging proteins, and proteins involved in DNA replication, transcription, and lysis; (ii) 49 hypothetical proteins; and (iii) seven proteins with no similarities in the databases (Supplementary Table S2). The genomic organization of PcaP2EGY was compared with its closely related Bacillus phage 1_ICo-2020 (Fig. 7 B). Unlike PcaP1EGY, ORF assignment to clusters I and II was difficult since information regarding genes in those clusters are lacking, especially the key genes coding for RNA polymerase and RNA ligase (Fig. 7 B, Supplementary Table S2).

3.5. Phylogenetic relationships between phages PcaP1EGY, PcaP2EGY and other related phages

Phylogenetic relationships were determined among phages PcaP1-EGY, PcaP2EGY, five other phages belonging to the family *Autographiviridae* including two type species (Pseudomonas phage phiKMV and Enterobacteria phage T7) and three phages related to phage PcaP1EGY (*Pectobacterium* phages Q19, vB_PcaP_P15_PC2B6, and Jarilo), as well as one phage related to phage PcaP2EGY (the unclassified phage Bacillus phage 1_ICo-2020) (Fig. 8). Phylogenetic trees generated using the deduced aa sequences of phage DNA polymerase (DNAP), RNA polymerase (RNAP), major capsid protein (MCP), and terminase (TER) all showed that phage PcaP1EGY is most closely related to Pectobacterium phage Q19, suggesting that PcaP1EGY belongs to the same species *Pektosvirus* as the Pectobacterium phage Q19 (Fig. 8). Since RNA polymerase was not annotated in the genomes of PcaP2EGY and the Bacillus phage 1_ICo-2020, these two phages were not included in the phylogenetic analysis based on RNA polymerase (Fig. 8B). Phage PcaP2EGY was found to be more closely related to Bacillus phage 1_ICo-2020, an unclassified phage of the class Caudoviricetes, than to PcaP1EGY and other six phages based on the phylogenetic trees derived from the predicted aa sequences of the phage DNA polymerase, major capsid protein and terminase (Fig. 8 A, C, D).

3.6. Lytic phages PcaP1EGY, PcaP2EGY, and their combination significantly reduced in vitro growth of P. carotovorum

Phages PcaP1EGY, PcaP2EGY and their 1:1 (v/v) combination were highly effective against *P. carotovorum* at all tested MOIs (1, 10 and 100) in a 12 h experiment and showed growth inhibition of *P. carotovorum* 4 h after phage inoculation (Fig. 9). When the culture of *P. carotovorum* was inoculated with the SM buffer without any of the phages, bacterial growth as measured by the absorbent value at OD_{600} continued to

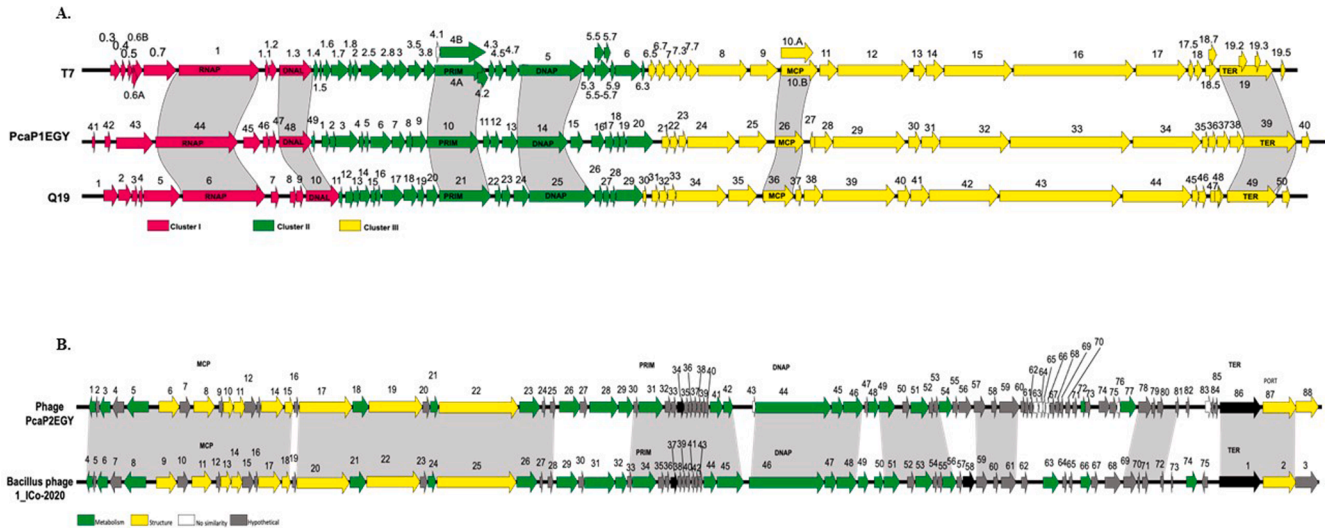


Fig. 7. Comparison of genomic organization between T7 phage and Pctobacterium phages PcaP1EGY and Q19 (A), and between Pectobacterium phage PcaP2EGY and Bacillus phage 1_ICo-2020 (B). The arrows represent the transcription direction of the annotated ORFs. The color codes for predicted gene functions in (A) are: red, early genes in functional clusters I; green, DNA metabolism in cluster II; and yellow, virion structure and assembly in cluster III. The color codes in (B) are: green, metabolism; yellow, structure; white, no similarity found in GenBank, and grey, hypothetical protein. Light grey shading connecting phages indicates ORFs with the same predicted functions. Numbers are annotated ORFs in each phage. RNAP, RNA polymerase; DNAP, DNA polymerase; MCP, major capsid protein; Ter, terminase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

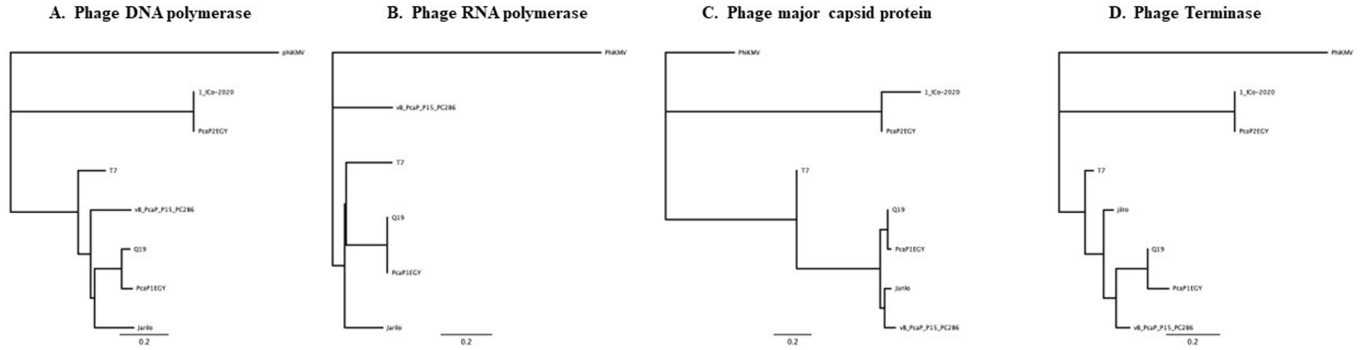


Fig. 8. Phylogenetic relationships among phages PcaP1EGY and PcaP2EGY and six other related phages, based on deduced amino acid sequences of phage DNA polymerase (A), RNA polymerase (B), major capsid protein (C), and terminase (D). The phylogenetic trees were generated using Geneious prime 2022 (https://www.geneious.com). Vertical distances are arbitrary, but the horizontal branches are proportional to genetic distance.

increase from 0.348 to 1.703, while inoculation with any of the phages significantly reduced the bacterial growth from the starting OD_{600} of 0.333 to a range of OD_{600} from 0.064 (PcaP1EGY + PcaP2EGY at MOI of 100) to 0.161 (PcaP1EGY + PcaP2EGY at MOI of 1) 4 h after phage inoculation (Fig. 9). In general, even though bacterial growth in the nine phage treatments increased slightly 6 to 12 h after incubation, the reduction in bacterial growth caused by the nine phage treatments was still significantly lower than the SM buffer control 12 h after incubation with the phages (Fig. 9).

3.7. Lytic phages PcaP1EGY, PcaP2EGY, and their combination significantly reduced soft rot symptoms caused by P. carotovorum on potato tubers

We observed that under both MOIs of 1000 and 10000, the average diameters of rotting tissue around the inoculation site in both cultivars of potato tubers were significantly smaller when the tubers inoculated with *P. carotovorum* were treated with the phages either singly or in combination, as compared to the tubers without any phage treatment, although PcaP1EGY or PcaP2EGY alone provided better protection for the tubers against soft rot than the combination of the two phages (Fig. 10). For potato cv. Cara tubers, at the MOI of 10^3, phage PcaP1EGY reduced tuber tissue maceration (soft rot symptoms) caused by *P. carotovorum* strain 100H by 68.30 % while phage PcaP2EGY decreased tissue maceration by at least 78.03 %, as compared to the pathogen control potato tubers without any phage treatment (Fig. 10, Supplementary Fig. 2). When the two phages were used together, they reduced tissue maceration by 51.24 % as compared to the pathogen only control (Fig. 10). For potato cv. Spunta tubers, at the MOI of 10^3, phage PcaP1EGY decreased tuber tissue maceration by 76.30 % while phage PcaP2EGY reduced tissue maceration by 73.69 % as compared to the pathogen only control (Fig. 10). When the two phages were combined, they reduced tissue maceration by 55.29 %. At the MOI of 10^4, PcaP1-EGY or PcaP2EGY used by itself completely prevented tissue maceration of potato tubers inoculated with *P. carotovorum* strain 100H (Fig. 10, Supplementary Fig. 2).

3.8. Phages PcaP1EGY and PcaP2EGY significantly reduced disease incidence caused by P. carotovorum strain 100H in potato plants grown from tubers in pots

Phages PcaP1EGY and PcaP2EGY significantly reduced disease incidence caused by *P. carotovotum* strain 100H in potato plants grown from tubers in pots (Figs. 11 and 12, Supplementary Fig. 3). Soil

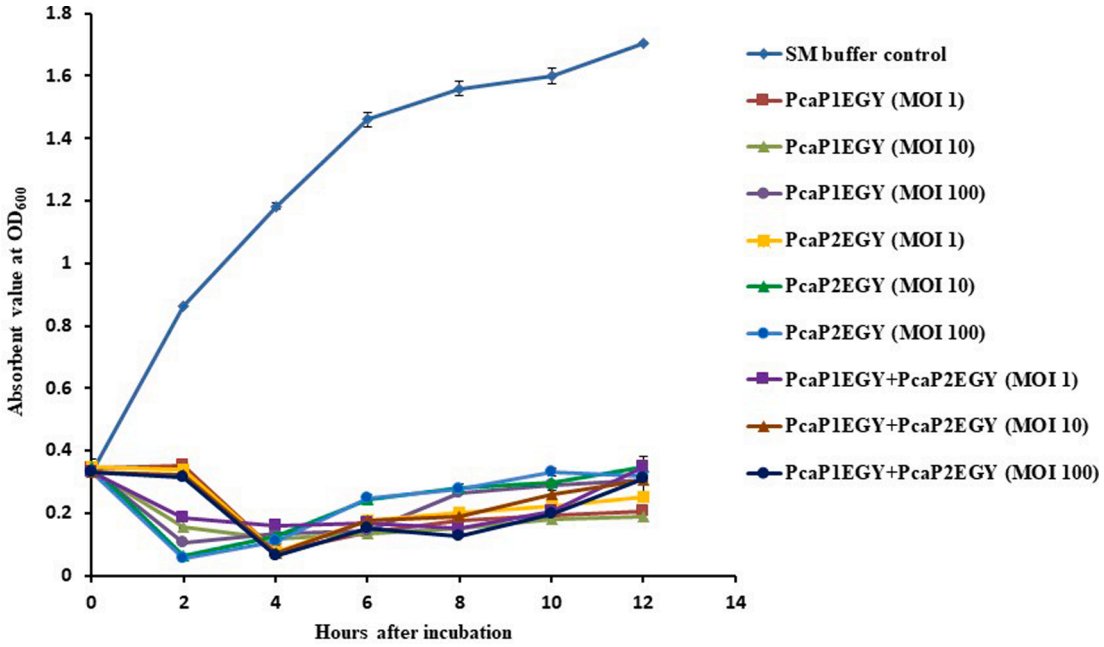


Fig. 9. Effect of phages PcaP1EGY, PcaP2EGY and their combination (PcaP1EGY:PcaP2EGY = 1:1) on *in vitro* growth of *P. carotovorum* at MOIs of 1, 10, and 100, respectively. Each value represents a mean of three replicates. Bars represent standard deviation.

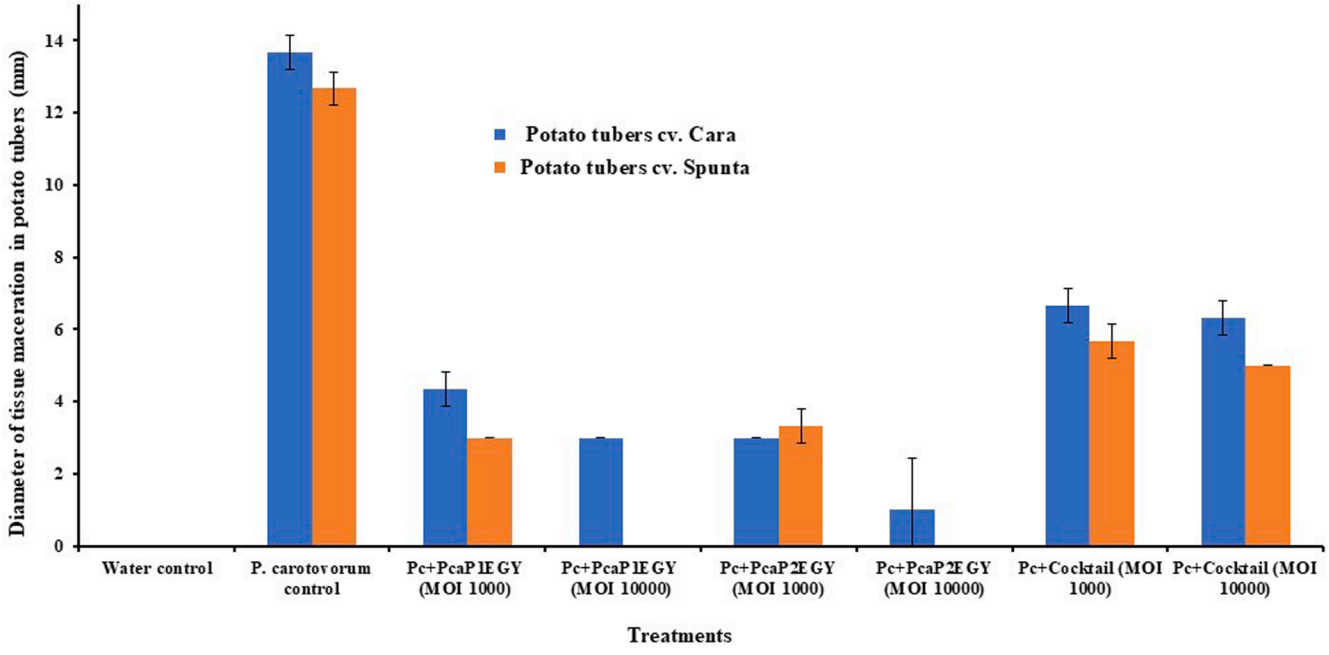


Fig. 10. Comparison of tissue maceration of potato tubers of cultivars Cara and Spunta inoculated with 100 μL of 24-h *P. carotovorum* (Pc) (2×10^8 CFU/mL) and treated with phages PcaP1EGY and PaP2EGY either singly or in 1:1 (v:v) combination (cocktail) at a MOI of 1000 or 10,000 72 h after inoculation and incubation at 28 °C, as compared to the controls without any phage treatment. Bars represent standard deviation.

drenching and foliar spray with either phage significantly reduced the disease incidence caused by *P. carotovotum* strain 100H from 97 % in the pathogen only control plants to 3 and 14 %, corresponding to treatment efficiency of 94 and 83 % in plants treated with PcaP1EGY and PcaP2-EGY, respectively (Fig. 11). Furthermore, treatment by either phage PcaP1EGY or PcaP2EGY resulted in a remarkable reduction in AUDPC (3 for PcaP1EGY treatment and 12 for PcaP2EGY treatment) compared to the pathogen control plants with the highest level of AUDPC (59.3) (Fig. 12). Disease development in the pathogen control plants inoculated only with *P. carotovotum* strain 100H was extremely rapid with plants showing disease symptoms (wilting, yellowing, stunting, black stem, and tissue maceration) two days after bacterial inoculation and completely wilted or dead 10 more days later. In *P. carotovorum*-inoculated and phage treated potato plants, limited disease symptoms were observed 9 days after bacterial inoculation in only two out of 10 plants with an average disease incidence of 14 %. Potato plants in the water control and two phage control treatments displayed no disease symptoms (Figs. 11 and 12). Furthermore, PcaP1EGY and PcaP2EGY significantly reduced the population of *P. carotovotum* collected from rhizosphere soil in the pots 30 and 70 days after infestation of the soil

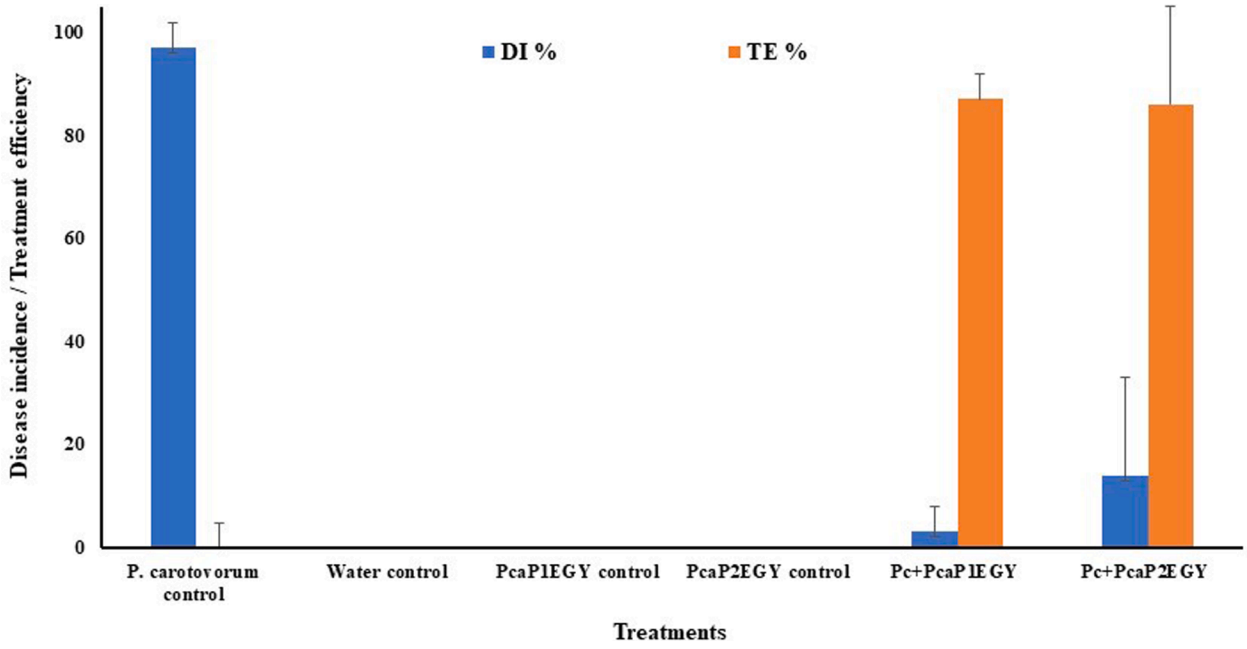


Fig. 11. Disease incidence caused by *P. carotovorum* in potato plants grown from tubers in pots and treatment efficiency by phage PcaP1EGY or PcaP2EGY calculated based on Kheirandish and Harighi (2015). All values represent means of 2 experiments, each containing 10 plants per treatment.

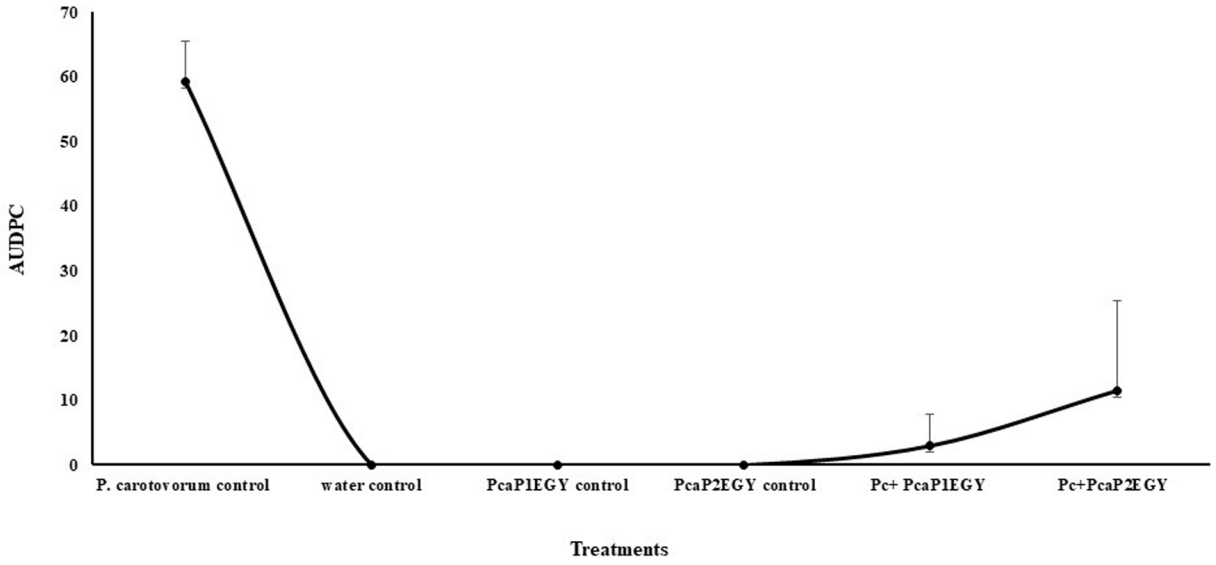


Fig. 12. Comparison of the area under the diseases progress curve (AUDPC) determined according to Ilicic et al. (2023) until all plants in the *P. carotovorum* control treatment were dead (10 days after inoculation of *P. carotovorum* by soil drenching).

with *P. carotovotum* (Fig. 13). By measuring potato yield for each treatment in the experiment, it was found that both phages PcaP1EGY and PcaP2EGY protected potato plants by maintaining the weight of potato tubers to the levels similar to the ones in the water control and the two phage only control treatments, as compared to potato tuber weight of 0 in plants inoculated with *P. carotovorum* without any phage treatment, since all plants were dead after initially showing wilt symptoms (Fig. 14, Supplementary Fig. 3).

4. Discussion

In this study, two different novel *P. carotovorum*-infecting phages PcaP1EGY and PcaP2EGY were isolated from potato-cultivating soil in Egypt. Both phages are lytic by producing clear plaques and have potential as biocontrol agents for *P. carotovorum*. Naligama and Halmillawewa (2022) isolated *P. carotovorum*–infecting phage vB_PcaM_P7_Pc from infected carrot samples with typical symptoms of soft rot obtained from storage areas at marketplaces in the Gampaha District of Sri Lanka. Czajkowski et al. (2015) screened for the presence of lytic phages against various *Pectobacterium* species by collecting samples of potato rhizosphere soil, bulk soil, potato stem, and tubers from arable potato fields in various regions of Poland. Phages PcaP1EGY and PcaP2EGY produced clear plaques with different sizes (2–3 mm for PcaP1EGY and 3–4 mm for PcaP2EGY). Other lytic *P. carotovorum*-infecting phages have been isolated from various sources, but with smaller plaque sizes, including six by Kim et al. (2022) with clear plaques from sewage water, phage vB_PcaM_P7_Pc by Naligama and Halmillawewa (2022) that produced small clear plaques of about 0.5 to 1

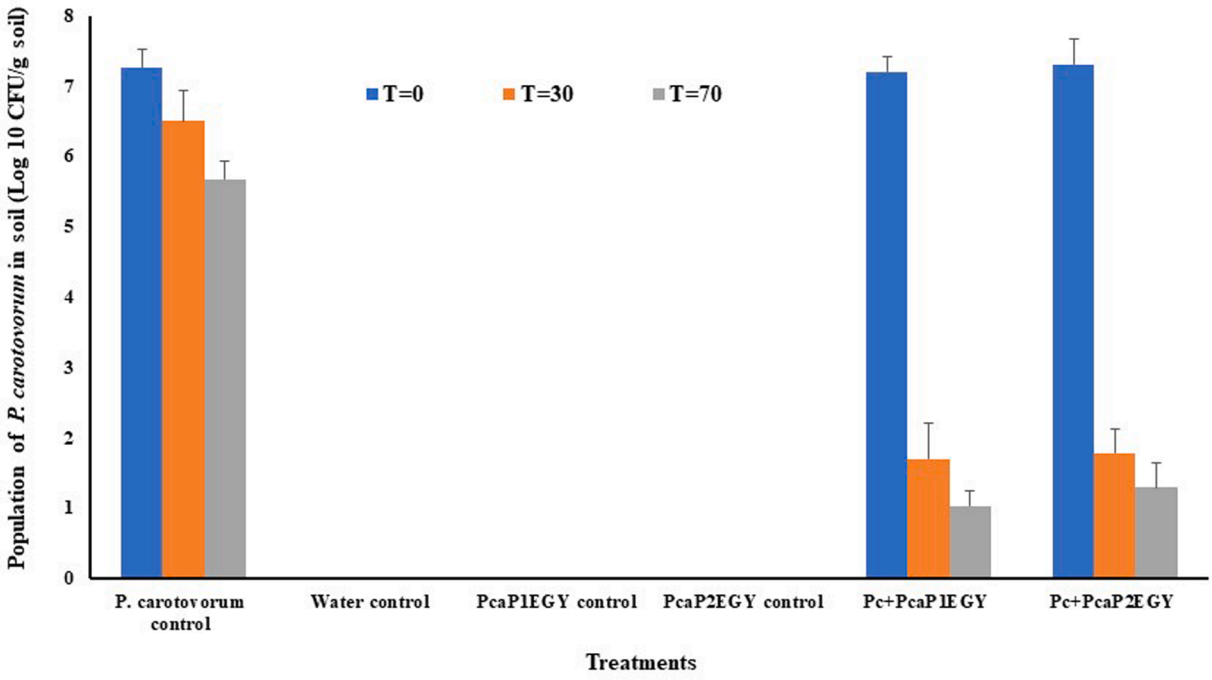


Fig. 13. Determination of *P. carotovorum* population in the rhizosphere soil in each treatment with the starting inoculation population of 3.2 x 10^7 CFU/g soil by soil drenching (T = 0), as well as 30 (T = 30) and 70 (T = 70) days after inoculation. All values represent means of 2 experiments, each containing 10 plants per treatment. Bars indicate standard deviation.

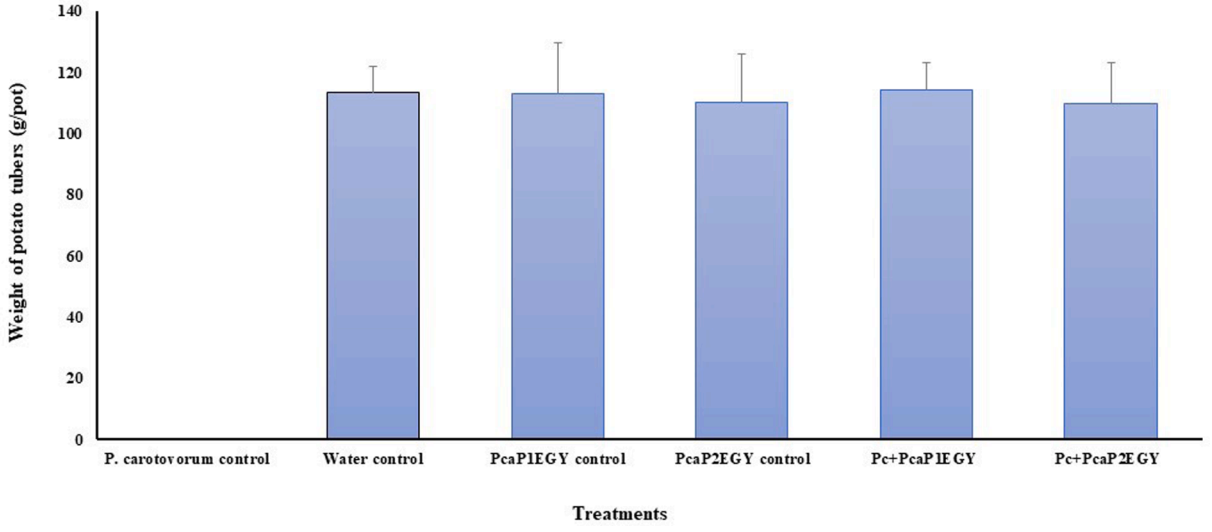


Fig. 14. Potato tuber weight measured 100 days after soil drenching inoculation with *P. carotovorum* strain 100H (Pc) and treated with or without phage PcaP1EGY or PcaP2EGY. All values represent means of 2 experiments, each containing 10 plants per treatment. Bars indicate standard deviation.

mm in diameter on nutrient agar overlay under laboratory conditions, and seven (Wc1, Wc2, Wc6, Wc7, Wc8, Wc9, and Wc10) from water and four (Wc3, Wc4, Wc5, and Wc5r) from soil samples by Muturi et al. (2019) in China that all produced small lytic plaques of about 1 mm in diameter.

Phages are very specific to the bacteria they infect; however, a few and rare cross-reactivity to different genera and species have been reported (Nasr-Eldin et al., 2023). Phages PcaP1EGY and PcaP2EGY were able to infect only the four *P. carotovorum* strains used in this study, suggesting that PcaP1EGY and PcaP2EGY are specific to *P. carotovorum* similar to *P. carotovorum* phage vB_PcaM_P7_Pc (Naligama and Halmillawewa, 2022). Some *Pectobacterium* phages (Jarilo, PP47 and Q19) have a relatively broad host range by infecting different species of *Pectobacterium* (Evseev et al., 2020; Pedersen et al., 2020) while others demonstrate a narrower one (Arno160, CB5) (Shneider et al., 2020; Buttimer et al., 2018a; Buttimer et al., 2018b). The SRP phage CB7 had a restricted host range, infecting only 5 out of 19 tested strains of *P. atrosepticum* (Buttimer et al., 2020). The specificity of phages only to their target bacteria makes it possible to maintain the native microbiota, especially those non-target bacteria that are potentially beneficial (Loc-Carrillo and Abedon, 2011).

PcaP1EGY and PcaP2EGY are similar to each other in morphology and host range, but different in genome size and genomic organization. Traditionally, phages are classified and named based on the morphology of purified virion particles observed under transmission electron microscope (Comeau et al., 2012; Ackermann, 2009; Ackermann, 1998).

As a result, tailed phages with long flexible non-contractile tails are classified as siphoviruses, with short expandable tails as podoviruses, and with long contractile tails as myoviruses. Since PcaP1EGY and PcaP2EGY have the characteristics of podoviruses with icosahedral heads and short tails, they belong to the order Caudovirales (Ackermann, 2012; Turner et al., 2021). This is similar to other reported *P. carotovorum*-infecting phages with morphological characteristics of podoviruses including vB_PcaP-A3, Arno160, vB_PatP_CB4, and phage Amaethon (Buttimer et al., 2018b; Shneider et al., 2020; Parena et al., 2022). Other phages with morphological features of podoviruses have also been reported for *Pectobacterium* spp.-infecting phages including four with heads of 50.2 nm x 51.3–56.29 nm x 54.86 nm and short non-contractile tails of up to 14.2 nm by Zaczek-Moczydłowska et al. (2020) and the PPc_A3 phage with an icosahedral head of 80 nm and a short non-contractile tail of 20 nm by Parena et al. (2022). Our complete genome sequence comparison and phylogenetic analysis revealed that phage PcaP1EGY belongs to the genus *Pektosvirus* in the class Caudoviricetes because of its close phylogenetic relationship with phage Q19, in agreement with the morphology results by electron microscopy. On the other hand, Phage PcaP2EGY is more closely related to Bacillus phage 1_ICo-2020, an unidentified phage of the class Caudoviricetes (Fig. 8). This suggests that PcaP2EGY might be a new member of a new genus together with the Bacillus phage 1_ICo-202.

Results from our one-step growth curve analysis revealed that phages PcaP1EGY and PcaP2EGY had a large burst size of 599 and 570 PFU/infected cell, respectively, with a much shorter latent period of 30 min. The burst size of phages PcaP1EGY and PcaP2EGY is larger than that of many other reported *P. carotovorum* phages (Czajkowski et al., 2015; Naligama and Halmillawewa, 2022). *Autographiviridae* phages are promising as biocontrol agents due to their virulent lifestyle, relatively short life cycle and large burst size (Miroshnikov et al., 2021). The shorter latent period has been related positively to effective inactivation of bacterial hosts (Li et al., 2020), indicating the likelihood of phages PcaP1EGY and PcaP2EGY against *P. carotovorum* in practical settings.

The stability of a phage under different environmental conditions is an important characteristic for selection of a phage for biocontrol. One essential factor affecting phage stability is the acidity or alkalinity of the environment. Our results indicate that phages PcaP1EGY and PcaP2EGY are viable at a wider range of pH from 3 to 11 over a period of 1 h than other *Pectobacterium* phages such as vB_PatM_CB7 which was viable at the pH of 5 to 11 (Buttimer et al., 2020), vB_PcaM_P7_Pc that survived a pH range of 4 to 10 (Naligama and Halmillawewa, 2022), and PP1 that was viable at the pH of 4 to 11, but completely inactivated at pH 3 and 12 (Lim et al., 2013).

Temperature plays a key role in phage survival, attachment, multiplication, and the length of the latent period (Olson et al., 2004). After incubation for one hour, phages PcaP1EGY and PcaP2EGY were found to be viable between the temperature range of 4 to 70 °C. *P. parmentieri*-infecting phages ϕA38 and ϕA41 were able to survive at the temperature of 85 °C for 24 h, but incubation under this temperature resulted in 100-fold reduction in phage numbers (Smolarska et al., 2018). Studies by Buttimer et al. (2020) and Parena et al. (2022) have shown that *Pectobacterium* phages retained infectivity only at 50 °C to 60 °C. The fact that phages PcaP1EGY and PcaP2EGY survived a wide temperature range of 4 to 70 °C suggests that both phages might efficiently infect *P. carotovorum* in a variety of temperatures under which *P. carotovorum* naturally persists in potato-associated environment.

In this study, we found that the titers of phages PcaP1EGY and PcaP2EGY were not significantly affected after exposure to UV irradiation for 10 min, although their titers were significantly reduced after longer exposure time. In contrast, UV irradiation for 10 min inactivated *Pectobacterium* phage φMA2 (Zaczek-Moczydłowska et al., 2020). *Pectobacterium* phages ϕA38 and ϕA41 were completely deactivated by exposure to UV irradiation for 5 min (Smolarska et al., 2018). Sunlight-mediated damage to phage particles is characterized by the formation of pyrimidine dimers (mostly thymine) in DNA caused by direct UV-light absorption (Ravanat et al., 2001).

Phages PcaP1EGY and PcaP2EGY were able to persist under different NaCl concentrations (2–10 %), suggesting that these phages may withstand high concentrations of salt. Our result agrees with a previous study by Smolarska et al. (2018) who reported that phage survival was not affected by incubation in solutions containing 0.05, 0.5 and 5.0 M NaCl.

Based on our results, since phages PcaP1EGY and PcaP2 EGY survived a wider range of temperature, pH and salt concentration conditions and a longer exposure time of UV irradiation than some other *Pectobacterium* phages, they have the potential as effective biocontrol agents under field application conditions.

In our study, we found that both phages (PcaP1EGY and PcaP2EGY), used either alone or in combination, not only significantly reduced *in vitro* growth of *P. carotovorum* under all tested MOIs of 1, 10 and 100, but also displayed remarkable protection against tissue maceration of potato tubers under MOIs of 1000 and 10000 on both potato cultivars of Cara and Spunta using our tuber maceration assay. In general, the level of protection by the phages was relatively higher in cultivar Spunta than Cara, which may be due to the higher susceptibility of cultivar Cara to infection by *P. carotovotum* than other cultivars including Spunta, as observed by the aggressive tuber maceration symptoms (rotted tissues) on the pathogen control tubers (phage-untreated) of cultivar Cara. Similarly, effective control against *P. carotvorum* using bioassays was reported by Voronina et al. (2019) showing that phage PP16 successfully slowed down the development of soft rot in potato tissue caused by *P. carotovorum* strain Pcc F002. Additionally, tissue maceration was reduced by 80 % in the slices and whole potato tubers after combined application of two lytic phages PD10.3 and PD23.1 (Czajkowski et al., 2015). A different study by Lim et al. (2013) using non-potato plants showed that phage PP1 exhibited an effective protection against *P. carotovorum* in lettuce.

Results from our greenhouse pot experiment revealed that both phages PcaP1EGY and PcaP2EGY possess remarkable suppressive capability against potato soft rot as indicated by significant reduction in disease severity and AUDPC of potato plants and the population level of *P. carotovorum* in rizhosphere soil even 70 days after soil infestation with *P. carotovorum*, as compared to the pathogen only control without any phage treatment. Furthermore, protection of potato plants by the phages lasted to the tuber formation stage by maintaining similar yield of potato tubers per pot as the one in the water control tubers. On the contrary, the pathogen control potato plants without any phage treatment displayed aggressive soft rot symptoms, rapid death, and a total loss of potato tuber yield with the soft rot symptoms appearing two days after soil infestation with *P. carotovorum* and complete death of potato plants 10 more days later. The facts that both phages PcaP1EGY and PcaP2EGY are lytic, survived a wide range of temperature, pH, salt concentration and UV irradiation conditions, and displayed effective protection against soft rot in both potato tubers and potato plants, as revealed by our study, suggest that both phages PcaP1EGY and PcaP2EGY have great potential as biocontrol agents against soft rot caused by *P. carotovorum*.

5. Conclusion

Two lytic *P. carotovorum*-specific phages PcaP1EGY and PcaP2EGY were isolated from clay soil in Egypt. Both phages have large burst size, tolerance for a wide range of temperature, pH, UV irradiation and salt concentration conditions, and effectively reduced the *in vitro* growth of *P. carotovorum* and soft rot on potato tubers and potato plants, suggesting that they have great potential as biocontrol agents to effectively control soft rot in potato caused by *P. carotovorum*. Phages PcaP1EGY and PcaP2EGY are also the first two *Podoviridae P. carotovorum*-infecting phages from Egypt with their complete genome sequences determined. Phylogenetic analysis indicates that phage PcaP1EGY belongs to the genus *Pektosvirus* in the family of *Autographiviridae*, while phage PcaP2EGY could potentially be assigned as a member of a new viral genus in the class Caudoviricetes with the unclassified Bacillus phage

1_ICo-2020.

Funding

Part of this study was implemented in the research plan of Bacterial Disease Research Department to control plant diseases under the umbrella of Agricultural Research center (ARC), Egypt.

CRediT authorship contribution statement

Kamel M. Elhalag: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **Mohamed A. Nasr-Eldin:** Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Conceptualization. **Qi Huang:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Formal analysis. **Abd-El-Aziz M. Rabab:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Abdelmonim Ali Ahmad:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to express their gratitude to the Plant Pathology Research Institute, ARC for facilitating research collaboration among different laboratories, and providing equipment and greenhouse to conduct most of the experiments in this study.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2024.105444.

References

Abedon, S. T., García, P., Mullany, P., and Aminov, R., 2017. Editorial: Phage Therapy: Past, Present and Future. Front. Microbiol. 8:981. doi: 10.3389/fmicb.2017.00981.

Abo-Elyousr, K.A.M., Sallam, M.A., Hassan, M.H., Allam, A.D., 2010. Effect of certain cultural practices on susceptibility of potato tubers to soft rot disease caused by Erwinia carotovora pv. carotovora. Arch. Phytopathol. Plant Protect. 43, 1625–1635. https://doi.org/10.1080/03235400902753576.

Ackermann, H.W., 1998. Tailed bacteriophages: the order Caudovirales. Adv. Virus Res. 51, 135–201. https://doi.org/10.1016/S0065-3527(08)60785-X.

Ackermann, H.W., 2009. Phage classification and characterization. Methods Mol. Biol. 501, 127–140. https://doi.org/10.1007/978-1-60327-164-6_13.

Ackermann, H.W., 2012. Bacteriophage electron microscopy. Adv. Virus Res. 82, 1–32. https://doi.org/10.1016/B978-0-12-394621-8.00017-0.

Adams, M.H., 1959. Bacteriophages. Wiley Interscience Publishers, New York, pp. 443–522.

Adeolu, M., Alnajar, S., Naushad, S., and Gupta, R. S., 2016. Genome-based phylogeny and taxonomy of the '*Enterobacteriales*': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and Budviciaceae fam. nov. Int. J. Syst. Evo. Microbiol. 66(12), 5575–5599. https://doi.org/10.1099/ijsem.0.001485.

Adriaenssens, E.M., van Vaerenbergh, J., Vandenheuvel, D., Dunon, V., Ceyssens, P.J., de Proft, M., et al., 2012. T4-related bacteriophage LIMEstone isolates for the control of soft rot on potato caused by "*Dickeya solani*". PLoS One 7, e33227. https://doi.org/10.1371/journal.pone.0033227.

Agyemang, P.A., Kabir, M.N., Kersey, C.M., Dumenyo, C.K., 2020. The bacterial soft rot pathogens, *Pectobacterium carotovorum* and *P. atrosepticum*, respond to different classes of virulence-inducing host chemical signals. Horticulturae 6 (1), 1–13. https://doi.org/10.3390/horticulturae6010013.

Ahmad, A.A., Stulberg, M.J., Mershon, J.P., Mollov, D.S., Huang, Q., 2017. Molecular and biological characterization of φRs551, a filamentous bacteriophage isolated from a race 3 biovar 2 strain of *Ralstonia solanacearum*. PLoS One 12, e0185034.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., et al., 1997. (Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25 (17), 3389–3402. https://doi.org/10.1093/nar/25.17.3389.

Armon, R., Kott, Y., 1993. A simple, rapid and sensitive presence/absence detection test for bacteriophage in drinking water. J. Appl. Bacteriol. 74, 490–496. https://doi.org/10.1111/j.1365-2672.1993.tb05159.x.

Aslam, B., Wang, W., Arshad, M.I., Khurshid, M., Muzammil, S., Rasool, M.H., et al., 2018. Antibiotic resistance: a rundown of a global crisis. Infect. Drug Resist. 11, 1645–1658. https://doi.org/10.2147/IDR.S173867.

Beňo, F., Horsáková, I., Kmoch, M., Petrzik, K., Krátká, G., Ševčík, R., 2022. Bacteriophages as a strategy to protect potato tubers against *Dickeya dianthicola* and *Pectobacterium carotovorum* soft ot. Microorganisms 10 (12), 2369. https://doi.org/10.3390/microorganisms10122369.

Besemer, J., 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res. 29, 2607–2618. https://doi.org/10.1093/nar/29.12.2607.

Bhat, K.A., Masood, S.D., Bhat, N.A., Bhat, M.A., Razvi, S.M., Mir, M.R., et al., 2010. Current status of post harvest soft rot in vegetables: a review. Asian J. Plant Sci. 9 (4), 200–208. https://doi.org/10.3923/ajps.2010.200.208.

Buttimer, C., Lucid, A., Neve, H., Franz, C. M., O'Mahony, J., Turner, et al., 2018b. *Pectobacterium atrosepticum* Phage VB_PatP_CB5: A member of the proposed genus '*Phimunavirus*'. Viruses 10:394. doi: 10.3390/v10080394.

Buttimer, C., Hendrix, H., Lucid, A., Neve, H., Noben, J.P., Franz, C., et al., 2018a. Novel N4-like bacteriophages of *Pectobacterium atrosepticum*. Pharmaceuticals (Basel) 11 (2), 45.

Buttimer, C., Lynch, C., Hendrix, H., Neve, H., Noben, J.P., Lavigne, R., Coffey, A., 2020. Isolation and characterization of Pectobacterium phage vB_PatM_CB7: new insights into the genus *Certrevirus*. Antibiotics 9, 352. https://doi.org/10.3390/antibiotics9060352.

Carstens, A.B., Djurhuus, A.M., Kot, W., Hansen, L.H., 2019. A novel six-phage cocktail reduces *Pectobacterium atrosepticum* soft rot infection in potato tubers under simulated storage conditions. FEMS Microbiol Lett. 366 (fnz101) https://doi.org/10.1093/femsle/fnz101.

Charkowski, A.O., 2018. The changing face of bacterial soft-rot diseases. Ann. Rev. Phytopathol. 56, 269–288. https://doi.org/10.1146/annurev-phyto-080417-045906.

Comeau, A.M., Tremblay, D., Moineau, S., Rattei, T., Kushkina, A.I., Tovkach, F.I., et al., 2012. Phage morphology recapitulates phylogeny: the comparative genomics of a new group of myoviruses. PLoS One 7, e40102.

Czajkowski, R., Pérombelon, M.C.M., van Veen, J.A., van der Wolf, J.M., 2011. Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review. Plant Pathol. 60, 999–1013. https://doi.org/10.1111/j.1365-3059.2011.02470.x.

Czajkowski, R., Ozymko, Z., Lojkowska, E., 2014. Isolation and characterization of novel soilborne lytic bacteriophages infecting Dickeya spp. biovar 3 ('D. solani'). Plant Pathol. 63, 758–772. https://doi.org/10.1111/ppa.12157.

Czajkowski, R., Ozymko, Z., de Jager, V., Siwinska, J., Smolarska, A., Ossowicki, A., et al., 2015. Genomic, proteomic and morphological characterization of two novel broad host lytic bacteriophages FPD10.3 and FPD23.1 infecting pectinolytic *Pectobacterium* spp. and *Dickeya* spp. PLoS One 10 (e0119812).

Czajkowski, R., Smolarska, A., Ozymko, Z., 2017. The viability of lytic bacteriophage ΦD5 in potato-associated environments and its effect on Dickeya solani in potato (Solanum tuberosum L.) plants. PLoS One 12 (e0183200). https://doi.org/10.1371/journal.pone.0183200. PMID: 28800363.

Dunn, J. J., Studier, F. W., and Gottesman, M., 1983. Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. J. Mol. Biol. 166, 477–535. https://doi. org/10.1016/S0022-2836(83)80282-4.

El-Dougdoug, N.K., Cucic, S., Abdelhamid, A.G., Brovko, L., Kropinski, A.M., Griffiths, M. W., et al., 2019. Control of *Salmonella newport* on cherry tomato using a cocktail of lytic bacteriophages. Int. J. Food Microbiol. 293, 60–71. https://doi.org/10.1016/j.ijfoodmicro.01.003.

Elhalag, K.M., Emara, H.M., Messiha, N.A.S., Elhadad, S.A., Abdallah, S.A., 2015. The relation of different crop roots exudates to the survival and suppressive effect of Stenotrophomonas maltophilia (PD4560), biocontrol agent of bacterial wilt of potato. J. Phytopathol. 163, 829–840. https://doi.org/10.1111/jph.12381.

Elhalag, K.M., Elbadry, N., Farag, S., Hagag, M., Hussien, A., 2020. Etiology of potato soft rot and blackleg diseases complex in Egypt. J. Plant Dis. Protec. 127, 855–871. https://doi.org/10.1007/s41348-020-00354-6.

Evseev, P.V., Lukianova, A.A., Shneider, M.M., Korzhenkov, A.A., Bugaeva, E.N., Kabanova, A.P., et al., 2020. Origin and evolution of *Studiervirinae* bacteriophages infecting *Pectobacterium*: horizontal transfer assists adaptation to new niches. Microorganisms 8, 1707. https://doi.org/10.3390/microorganisms8111707.

Fahy, P.C., Hayward, A.C., 1983. Media and methods for isolation and diagnostic test. In: Fahy, P.C., Persley, G.J. (Eds.), Plant Bacterial Diseases: A Diagnostic Guide. (Academic Press, San Diego, CA, USA), pp. 337–378.

FAO FAOSTAT., 2022. Production and trade statistics. Available online: http://www.fao.org/faostat/en/#data/QC/visualize.

Farag, S.M.A., Elhalag, K.M.A., Hagag, M.H., Khairy, A.M., Ibrahim, H.M., Saker, M.T., et al., 2017. Potato bacterial wilt suppression and plant health improvement after application of different antioxidants. J. Phytopathol. 165, 522–537.

Gardan, L., Gouy, C., Christen, R., and Samson, R., 2003. Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov.,

Pectobacterium betavasculorum sp. nov. and *Pectobacterium wasabiae* sp. nov. Int. J. Syst. Evo. Microbiol. 53(2), 381–391. https://doi.org/10.1099/ijs.0.02423-0.

Garvey, M., 2022. Bacteriophages and food production: biocontrol and bio-preservation options for food safety. Antibiotics (Basel) 11 (10), 1324. https://doi.org/10.3390/antibiotics11101324.

Hammad, A. M., Zahran, H. H., Ahmad, M. S., and Ragab, A. l., 2016. Isolation and characterization of bacteriophages specific to root nodule bacterium *Rhizobium leguminosarum* bv. *viciae.* J. Basic. Appl. Sci. Res. 6(9), 17-25.

Haverkort, A.J., Struik, P.C., 2015. Yield levels of potato crops: Recent achievements and future prospects. Field Crops Res. 182, 76–85. https://doi.org/10.1016/j.fcr.2015.06.002.

Hayward, A.C. (2000). "*Ralstonia solanacearum*," In Encyclopedia of Microbiology, ed. L. Lederberg (Academic Press, San Diego), 32-42.

Ilicic, R., Jelušic, A., Milovanovic, P., Stankovic, S., Zecevic, K., Stanisavljevic, R., et al., 2023. Characterization of *Xanthomonas arboricola* pv. *pruni* from *Prunus* spp. orchards in western Balkans. Plant Pathol. 72 (2), 290–299. https://doi.org/10.1111/ppa.13658.

Jee, S., Choi, J.-G., Lee, Y.G., Kwon, M., Hwang, I., Heu, S., 2020. Distribution of *Pectobacterium* species isolated in South Korea and comparison of temperature effects on pathogenicity. Plant Pathol. J. 36, 346–354. https://doi.org/10.5423/PPJ.OA.09.2019.0235.

Kalischuk, M., Hachey, J., Kawchuk, L., 2015. Complete genome sequence of phytopathogenic *Pectobacterium atrosepticum* bacteriophage Peat1. Genome Announc. 3 (4), 1–2. https://doi.org/10.1128/genomeA.00760-15.

Kawasaki, T., Shimizu, M., Satsuma, H., Fujiwara, A., Fujie, M., Usami, et al., 2009. Genomic characterization of *Ralstonia solanacearum* phage φRSB1, a T7-like wide-host-range phage. J. Bacteriol. 191, 422–427. https://doi.org/10.1128/JB.01263-08.

Kheirandish, Z., Harighi, B., 2015. Evaluation of bacterial antagonists of *Ralstonia solanacearum*, causal agent of bacterial wilt of potato. Biol. Control. 86, 14–19. https://doi.org/10.1016/j.biocontrol.2015.03.007.

Kim, H., Kim, M., Jee, S.N., Heu, S., Ryu, S., 2022. Development of a bacteriophage cocktail against *Pectobacterium carotovorum* subsp. *carotovorum* and its effects on *Pectobacterium* virulence. Appl. Environ. Microbiol. 88 (19), e0076122.

King, A. M., Lefkowitz, E., Adams, M. J., and Carstens, E. B., 2011. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier: San Diego, CA, USA, Volume 9.

King, E., Ward, M., Raney, D., 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44, 301–307.

Kropinski, A.M., Mazzocco, A., Waddell, T.E., Lingohr, E., Johnson, R.P., 2009. Enumeration of bacteriophages by double agar overlay plaque assay. Methods Mol. Biol. 501, 69–76. https://doi.org/10.1007/978-1-60327-164-6_7.

Lai, M.J., Lin, N.T., Hu, A., Soo, P.C., Chen, L.K., Chen, L.H., et al., 2011. Antibacterial activity of Acinetobacter baumannii phage ϕAB2 endolysin (LysAB2) against both gram-positive and gram-negative bacteria. Appl. Microbiol. Biotechnol. 90 (2), 529–539. https://doi.org/10.1007/s00253-011-3104-y.

Lavigne, R., Burkal'tseva, M. V., Robben, J., Sykilinda, N. N., Kuro- chkina, L. P., Grymonprez, B., et al., 2003. The genome of bacteriophage φKMV, a T7-like virus infecting *Pseudomonas aeruginosa. Virol.* 312, 49–59. https://doi.org/10.1016/S0042-6822(03)00123-5.

Lee, D.H., Lee, J.H., Shin, H., Ji, S., Roh, E., Jung, K., et al., 2012. Complete genome sequence of *Pectobacterium carotovorum* subsp. *carotovorum* bacteriophage My1. J. Virol. 86, 11410–11411. https://doi.org/10.1128/JVI.01987-12.

Lee, J.H., Shin, H., Ji, S., Malhotra, S., Kumar, M., Ryu, S., et al., 2012. Complete genome sequence of phytopathogenic *Pectobacterium carotovorum* subsp. *carotovorum* bacteriophage PP1. J. Virol. 86 (16), 8899–8900. https://doi.org/10.1128/JVI.01283-12.

Li, Z., Ma, W., Li, W., Ding, Y., Zhang, Y., Yang, Q., et al., 2020. A broad-spectrum phage controls multidrug-resistant Salmonella in liquid eggs. Food Res. Int. 132, 109011 https://doi.org/10.1016/j.foodres.2020.109011.

Lim, J.A., Jee, S., Lee, D.H., Roh, E., Jung, K., Oh, C., et al., 2013. Biocontrol of *Pectobacterium carotovorum* subsp. *carotovorum* using bacteriophage PP1. J. Microbiol. Biotechnol. 23 (8), 1147–1153. https://doi.org/10.4014/jmb.1304.04001.

Lim, J.A., Shin, H., Lee, D.H., Han, S.W., Lee, J.H., Ryu, S., Heu, S., 2014. Complete genome sequence of the *Pectobacterium carotovorum* subsp. *carotovorum* virulent bacteriophage PM1. Arch. Virol. 159 (8), 2185–2187. https://doi.org/10.1007/s00705-014-2005-7.

Lim, J.A., Lee, D.H., Heu, S., 2015. Isolation and genomic characterization of the T4-like bacteriophage PM2 infecting *Pectobacterium carotovorum* subsp. *carotovorum*. Plant Pathol. J. 31 (1), 83–89. https://doi.org/10.5423/PPJ.NT.09.2014.0099.

Lim, J.A., Heu, S., Park, J., Roh, E., 2017. Genomic characterization of bacteriophage vB_PcaP_PP2 infecting *Pectobacterium carotovorum* subsp. *carotovorum*, a new member of a proposed genus in the subfamily *Autographivirinae*. Arch. Virol. 162 (8), 2441–2444. https://doi.org/10.1007/s00705-017-3349-6.

Loc-Carrillo, C., Abedon, S.T., 2011. Pros and cons of phage therapy. Bacteriophage 1 (2), 480. https://doi.org/10.4161/bact.1.2.14590.

Lukianova, A.A., Shneider, M.M., Evseev, P.V., Shpirt, A.M., Bugaeva, E.N., Kabanova, A. P., et al., 2020. Morphologically different *Pectobacterium brasiliense* bacteriophages PP99 and PP101: deacetylation of O-polysaccharide by the tail spike protein of phage PP99 accompanies the infection. Front. Microbiol. 10, 3147. https://doi.org/10.3389/fmicb.2019.03147.

Mantsebo, C.C., Mazarura, U., Goss, M., Ngadze, E., 2014. The epidemiology of Pectobacterium and Dickeya species and the role of calcium in postharvest soft rot infection of potato (Solanum tuberosum) caused by the pathogens: A review. Afr. J. Agric. Res. 9 (19), 1509–1515.

Mihara, T., Nasr-Eldin, M.A., Chatchawankanphanich, O., Bhunchoth, A., Phironrit, N., Kawasaki, T., et al., 2016. A phage ϕRP15 is closely related to and encodes 19 tRNA-related sequences. Virol. Rep. 6, 61–73. https://doi.org/10.1016/j.virep.2016.07.001.

Miroshnikov, K.A., Evseev, P.V., Lukianova, A.A., Ignatov, A.N., 2021. Tailed lytic bacteriophages of soft rot Pectobacteriaceae. Microorganisms 9 (9), 1819. https://doi.org/10.3390/microorganisms9091819.

Muturi, P., Yu, J., Maina, A.N., Kariuki, S., Mwaura, F.B., Wei, H., 2019. Bacteriophages isolated in China for the control of *Pectobacterium carotovorum* causing potato soft rot in Kenya. Virol. Sin. 34 (3), 287–294. https://doi.org/10.1007/s12250-019-00091-7.

Nabhan, S., De Boer, S.H., Maiss, E., Wydra, K., 2013. *Pectobacterium aroidearum* sp. nov., a soft rot pathogen with preference for monocotyledonous plants. Int. J. Syst. Evo. Microbiol. 63 (Pt_7), 2520–2525. https://doi.org/10.1099/ijs.0.046011-0.

Naligama, K.N., Halmillawewa, A.P., 2022. *Pectobacterium carotovorum* phage vB_PcaM_P7_Pc is a new member of the genus *Certrevirus*. Microbiol. Spectr. 10 (6), e0312622. https://doi.org/10.1128/spectrum.03126-22.

Narváez-Barragán, D.A., Tovar-Herrera, O.E., Torres, M., Rodríguez, M., Humphris, S., Toth, I.K., Segovia, L., Serrano, M., Martínez-Anaya, C., 2020. Expansin-like Exl1 from *Pectobacterium* is a virulence factor required for host infection, and induces a defence plant response involving ROS, and jasmonate, ethylene and salicylic acid signalling pathways in *Arabidopsis thaliana*. Sci Rep 10 (1), 7747. https://doi.org/10.1038/s41598-020-64529-9.

Nasr-Eldin, M.A., Gamal, E., Hazza, M., et al., 2023. Isolation, characterization, and application of lytic bacteriophages for controlling Enterobacter cloacae complex (ECC) in pasteurized milk and yogurt. Folia Microbiol. 68, 911–924. https://doi.org/10.1007/s12223-023-01059-7.

Parena, A.J.S., Silva, B.B.I., Mercado, R.M.L., Sendon, A.A.A., Signabon, F.B., Balidion, J. F., et al., 2022. Lytic phages displayed protective effects against soft rot-causing *Pectobacterium* sp. Biocontrol Sci. Tech. 32, 1326–1345.

Pedersen, J.S., Carstens, A.B., Djurhuus, A.M., Kot, W., Neve, H., Hansen, L.H., 2020. *Pectobacterium* phage Jarilo displays broad host range and represents a novel genus of bacteriophages within the family *Autographiviridae*. PHAGE 1, 237–244. https://doi.org/10.1089/phage.2020.0037.

Pérombelon, M.C.M., 2002. Potato diseases caused by soft rot erwinias: an overview of pathogenesis. Plant Pathol. 51 (1), 1–12. https://doi.org/10.1046/j.0032-0862.2001.Shorttitle.doc.x.

Portier, P., Pédron, J., Taghouti, G., Fischer-Le Saux, M., Caullireau, E., Bertrand, C., et al., 2019. Elevation of *Pectobacterium carotovorum* subsp. *odoriferum* to species level as *Pectobacterium odoriferum* sp. nov., proposal of *Pectobacterium brasiliense* sp. nov. and *Pectobacterium actinidiae* sp. nov., emended description of *Pectobacterium carotovorum* and description of *Pectobacterium versatile* sp. nov., isolated from streams and symptoms on diverse plants. Int. J. Syst. Evo. Microbiol. 69 (10), 3207–3216. https://doi.org/10.1099/ijsem.0.003611.

Pritchard, L., Glover, R.H., Humphris, S., Elphinstone, J.G., Toth, I.K., 2016. Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. Anal. Methods 8, 12–24.

Rabia, A., Mohamed, A., Abdelaty, E., Metwally, S., Yacout, D., 2021. Investigating adaptation strategies developed by potato farmers to cope with climate change impacts in Egypt. Alexandria Sci. Exchange J. 42, 871–881. https://doi.org/10.21608/asejaiqjsae.2021.205325.

Rahman, M. M., Ali, M. E., Khan, A. A., Akanda, A. M., Uddin, Md. Kamal, Hashim, U., and Abd Hamid S. B. 2012. Isolation, characterization, and identification of biological control agent for potato soft rot in Bangladesh. The Scientific World Journal. doi:10.1100/2012/723293.

Ramirez, K., Cazarez-Montoya, C., Lopez-Moreno, H.S., Campo, N.-C.-D., 2018. Bacteriophage cocktail for biocontrol of *Escherichia coli* O157:H7: stability and potential allergenicity study. PLoS One 13, e0195023.

Rashid, A., Fahad, M.A.B., Khan, M.A., Mateen, A., 2012. Incidence of potato blackleg caused by *Pectobacterium atrosepticum* in district chiniot and its management through bio-products. Afr. J. Agric. Res. 7 (45), 6035–6048. https://doi.org/10.5897/AJAR12.1697.

Ravanat, J.L., Douki, T., Cadet, J., 2001. Direct and indirect effects of UV radiation on DNA and its components. J. Photochem. Photobiol. B 63, 88–102.

Shneider, M.M., Lukianova, A.A., Evseev, P.V., Shpirt, A.M., Kabilov, M.R., Tokmakova, A.D., et al., 2020. Autographivirinae bacteriophage Arno 160 infects *Pectobacterium carotovorum* via depolymerization of the bacterial O-polysaccharide. Int. J. Mol. Sci. 21 (9), 3170. https://doi.org/10.3390/ijms21093170.

Smolarska, A., Rabalski, L., Narajczyk, M., Czajkowski, R., 2018. Isolation and phenotypic and morphological characterization of the first Podoviridae lytic bacteriophages A38 and A41 infecting Pectobacterium parmentieri (former Pectobacterium wasabiae). Eur. J. Plant Pathol. 150, 413–425.

Svircev, A., Roach, D., Castle, A., 2018. Framing the future with bacteriophages in agriculture. Viruses 10, 218. https://doi.org/10.3390/v10050218.

Tolessa, E.S., 2018. Importance, nutrient content and factors affecting nutrient content of potato. Am. J. Food Nutr. Health 3 (3), 37–41.

Toth, I.K., Bell, K.S., Holeva, M.C., Birch, P.R.J., 2003. Soft rot erwiniae: from genes to genomes. Mol. Plant Pathol. 4, 17–30. https://doi.org/10.1046/j.1364-3703.2003.00149.x.

Toth, I. K., Barny, M., Brurberg, M. B., Condemine, G., Czajkowski, R., Elphinstone, J. G., et al., 2021. "*Pectobacterium* and *Dickeya*: environment to disease development," in Plant Diseases Caused by Dickeya and Pectobacterium Species, eds. F. Van Gijsegem, J. M. van der Wolf, and I. K. Toth (Springer International Publishing: Cham, Switzerland), 39–84.

Toth, I.K., van der Wolf, J.M., Saddler, G., Lojkowska, E., Helias, V., Pirhonen, M., et al., 2011. Dickeya species: An emerging problem for potato production in Europe. Plant Pathol. 60 (3), 385–399. https://doi.org/10.1111/j.1365-3059.2011.02427.x.

Turner, D., Kropinski, A.M., Adriaenssens, E.M., 2021. A roadmap for genome-based phage taxonomy. Viruses 13, 506. https://doi.org/10.3390/v13030506.

Voronina, M.V., Bugaeva, E.N., Vasiliev, D.N., Kabanova, A.P., Barannik, A.P., Shneider, M.M., et al., 2019. Characterization of *Pectobacterium carotovorum* subsp. *carotovorum* bacteriophage PP16 prospective for biocontrol of potato soft rot. Microbiol. 13, 458–469. https://doi.org/10.1134/S0026261719040118.

Zaczek-Moczydłowska, M. A., Young, G. K., Trudgett, J., Plahe, C., Fleming, C. C., Campbell, K., et al., 2020. Phage cocktail containing *Podoviridae* and *Myoviridae* bacteriophages inhibits the growth of *Pectobacterium* spp. under in vitro and in vivo conditions. PLoS One 15(4):e0230842. doi: 10.1371/journal.pone.0230842.

Zhang, Y., Fan, Q., Loria, R., 2016. A re-evaluation of the taxonomy of phytopathogenic genera *Dickeya* and *Pectobacterium* using whole genome sequencing data. Syst. Appl. Microbiol. 39, 252–259. https://doi.org/10.1016/j.syapm.2016.04.001.