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RESEARCH ARTICLE

Influence of some chemicals and solvents on the lytic activity and the adsorption of bacteriophages on *Pectobacterium carotovoroum* subsp. *carotovorum*

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Abstract

Recently, bacteriophages have been used to control hazardous bacterial soft rot disease on crops. However, agricultural plants are frequently treated with different chemicals (fertilizers, pesticides and solvents), so we assessed the effect of some commonly used chemicals and solvents on the lytic activity of tested bacteriophages and their adsorption potential. This study reports the isolation of three specific phages against the *Pectobacterium carotovorum* subsp. *carotovorum* DSM 30170 strain, designated as ϕPC1, ϕPC2 and ϕPC3, then partially characterized using electron microscopy and genome size. The 3 isolated phages belong to the Myoviridae family. The results obtained were based on the plaque-forming unit observed after incubation. By increasing the chemical concentrations (from 0.1 to 0.5 mM), calcium chloride ($CaCl_2$) and potassium chloride (KCl) showed a significant increase in the lytic activity of the phages. Copper sulphate ($CuSO_4$) and copper chloride ($CuCl_2$) showed a substantial decrease in the activity of ϕPC3; however, such a decrease was insignificant for ϕPC1 and ϕPC2. By increasing the solvent concentrations (from 30 % v/v to 70 % v/v), propanol, ethanol and methanol showed a significant decrease in the count of the three isolated phages, ϕPC1, ϕPC2 and ϕPC3, compared to the control. Chloroform was the only solvent that did not reduce the phage titer. Our findings offer significant information for developing a strategy to combat the *P. carotovorum* subsp. *carotovorum* caused bacterial soft rot disease. avoiding copper compounds and alcoholic solvents such as propanol, ethanol and methanol in plots where phages are applied seems advisable.

Keywords

Pectobacterium carotovorum; soft rot; bacteriophages; pesticides; fertilizers

Introduction

Numerous commercially significant vegetables, including carrot, cucumber, cabbage, eggplant, garlic, pepper, potato, onion, radish, squash, sweet potato and tomato, are susceptible to the destructive soft rot disease caused by *P. carotovorum* subsp. *carotovorum* (Pcc). At any point along the agricultural production cycle, including during planting, the growth season, transportation and storage, the diseases can be highly destructive and result in catastrophic losses. (Pcc), the most common soft rot pathogen, is a gram nega-

tive, necrotrophic, facultative anaerobe of the family *Enterobacteriaceae,* motile via flagella. The symptoms of bacterial soft rot include fast softening of the tuber tissues which could be reduced to a watery mass within a week under humid conditions and optimum temperatures.

The pathogen invades the roots through stomata, lenticels, wounds and the stolen mother plant, then multiplies quickly causing maceration of tuber tissues by secretion of different extracellular degrading enzymes (4). The ability of *Pectobacterium* species to produce and secrete large amounts of a variety of extracellular plant cell wall-degrading enzymes (PCWDE), such as pectate lyases (Pel), polygalacturonases (Peh), proteases (Prt) and cellulases (Cel), which cause extensive tissue maceration, rotting and ultimately the death of the entire plant, is essential to their virulence and pathogenicity (5). These bacterial rots cause extensive damage to crops in agriculture and in the potato sector, a total of EUR 46 million of annual losses in the European Union (6).

Phages, viruses that attack bacteria, are believed to play a significant ecological role in regulating bacterial activity and population. They represent a potentially significant biotic component that could impact bacterial soil populations and agricultural output. Phages infect their host through a range of biochemically diverse host surface receptors, such as carbohydrates, lipopolysaccharides and proteins. The phage host range is usually based on how precisely it interacts with the host receptor. For example, if the phage recognises a very specific region, it may only be able to infect a single host species or strain (8). When the lytic phage genome is injected into its host, it initiates processes that hijack the host metabolic systems to ultimately produce multiple viral progenies, which are then released from the host utilizing phage encoded lytic enzymes. These progenies are able to infect new hosts and repeat the lytic cycle. In the right circumstances, they can proliferate in the field and possibly achieve a level of control that chemicals cannot.

The use of bacteriophages in agriculture has many advantages, but their high specificity is the most significant. Because they cannot infect humans or animals, their usage in food is safe. They are persistent as long as their hosts are around and they multiply effectively in the environment. They do not need to be used repeatedly due to the population's rapid growth (12).

Chemicals used as insecticides, fertilizers and solvents significantly rely on agricultural productivity. Intentional releases of pesticides into the environment are made to eradicate weeds, insects, plant diseases and other pests that negatively impact farm or livestock output. Fertilizers are supplementary materials that are given to crops to boost productivity. Farmers use these daily to increase crop productivity. Solvents make sure that these compounds (fertilizers) dry precisely when needed and are efficiently applied to the desired crops. This guarantees that the least product is required to ensure food security.

Previous studies discussed the effect of agricultural chemicals (agrichemicals) on phages' lytic activity (infectivity) and their adsorption on their bacterial hosts. The changes in phage titer were seen *in vitro* (16) after mixing phages into fresh formulations of some of the most widely used agrochemicals. Only one of the tested chemicals, Kocide 2000 (active component copper-hydroxide), decreased the phage titer. It was found that copper sulphate and sodium chloride showed a significant increase in the activity of *Streptomyces* phages infecting *Streptomyces scabies,* causing potato scab disease (17).

To the best of our knowledge, however, no research discusses the impact of agrichemicals and solvents on the lytic activity and the adsorption of bacteriophages on Pcc. The main objective of the study was to find out how some commonly used agrichemicals affected the ability of Pcc phages to break down cells and attach to the Pcc DSM 30170 strain.

Materials and Methods

Bacterial strains and culture conditions

Ain Shams University in Egypt's Faculty of Agriculture provided the Pcc DSM 30170 strain. The bacterial cells were grown at 28 °C with shaking at 200 to 300 rpm in a Casamino acid Peptone Glucose (CPG) medium that contained 0.1% Casamino Acids, 1% peptone and 0.5% glucose.

Isolation and characterization of bacteriophages

Sampling and isolation of bacteriophages

Bacteriophages were isolated according to standard procedure (18) with some modifications from sewage water samples collected from a Sewage water treatment plant in Gabal Asfar, Khanka, Qalubeia, Egypt. For phage enrichment, 5 mm of mixed sewage water samples were put on hold in 50 ml of CPG medium previously inoculated with Pcc DSM 30170 strain with a 2×10^8 CFU/ml. The inoculated flasks were incubated for 24 h at 28 °C and 150 rpm in a shaker incubator (VIS-180, Taisite Lab Sciences Inc., USA). Centrifugation was performed on the resultant cell suspension for 15 min. at 6000 rpm. The supernatant was filtered through a membrane filter with a 0.22 µm pore size. The plaque assay was carried out using the double-layer agar (DLA) overlay method. 200 µl each of the log phase host bacteria Pcc DSM 30170 strain and the phage filtrate were combined with 0.3-0.4% soft agar and put onto a 1.5% hard CPG agar plate. For 24 h, the plates were incubated at 28 °C. After incubation, the plaques' size, shape and number were noted.

Preparation of the phage lysates

By using the single plaque isolation approach, phage lysates were created (19). Different plaques were chosen and added to 3 ml of the liquid culture of the Pcc DSM 30170 strain based on the morphological shape of the plaques' characters. The cultures were incubated for 24 h at 150 rpm per min and 28 °C. To get the progeny phages, the cells were centrifuged. At least 3 repetitions were required to complete the procedure to achieve homogeneous plaque morphology.

Propagation of phages

A large amount of high titer phage stock was generated by propagating the phage isolates on their liquid Pcc DSM 30170 strain culture. A single plaque was chosen and added to an Pcc DSM 30170 log phase culture (24-h culture). The phage-host mixture was incubated at 28 °C for 24 h before being centrifuged in a chilled centrifuge (INO-BRC Micro, INOVIA technology, Turkey) for 10 min at 6000 rpm. The phage particles were then precipitated in the presence of 0.5 M NaCl and 5% polyethylene glycol 6000 after the filtrate had been passed through a membrane filter with a 0.22-µm pore size. Moreover, SM buffer, including 100 mM NaCl, 10 mM $MgSO_4$ and 0.01% gelatin, was used to dissolve the pellet after it had been centrifuged at 6000 rpm for 30 min at 4 °C. Phages that had been purified were stored at 4 °C until use (20).

Transmission electron microscopy

On 200 mesh carbon-coated copper grids, a drop of (10^{12} pfu/mL) phage suspension was placed, and the excess was drawn off with filter paper as described (21). Next, the grids were covered with a 2% uranyl acetate solution and the excess was drawn off as before and then examined using a transmission electron microscope (Model JEOL JEM- 2100) at Nano Tech Egypt, Dreamland, 6th October, Cairo, Egypt.

Phages DNA extraction

Each phage's nucleic acid was extracted using a modified phenol/chloroform technique (22). Bacteriophage suspension in a volume of 947.5 µL was gently combined with 2.5 µL of proteinase K (20 mg. mL^{-1}) and 50 µL of 10% (w/v) SDS and then incubated for one h at 56 °C. The solution was then topped off with an equal volume of 25:24:1 phenol-chloroform-isoamyl alcohol (Merck, UK). The liquid was thoroughly mixed before being centrifuged at room temperature for 15 min at 12000 g. The aqueous phase was gathered. This phenol-chloroform-isoamyl alcohol extraction step was repeated twice. Nucleic acids precipitated in ice-cold 70% ethanol and the pellet were resuspended in 50 µl of TE buffer pH 8 and stored at -20 °C.

DNA Quantification

A spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific) was used to measure the nucleic acid concentration and agarose gel 1% electrophoresis was used to verify the integrity of the nucleic acids.

Phages DNA digestion with restriction enzymes

A mixture of *Hind*III (10 U/L) and *Eco*RI (10 U/L) was utilized for DNA fragmentation as per the manufacturer's protocol (Thermo Fisher Scientific). Also used as a DNA ladder was Quick-Load® Purple 2-log DNA Ladder (0.1 - 10.0 kb) - New England Biolabs, Inc.: N0550S. Finally, data interpretation was done using Totallab (Ver.1.1) software. MultiSUB Maxi, Maxi Horizontal Electrophoresis System Cleaver Scientific was used for fragmentation. DNA fragment sizes derived from restriction enzyme digestion profiles were used to determine the phage genome size.

Thermal stability of phages

The thermal inactivation point of Pcc bacteriophages *in vitro* was carried out with some modifications (24). Phages suspensions were incubated at various temperatures for 10 min: 30, 40, 50, 60, 70, 80 and 90 °C. As previously described, the plaque assay was carried out 3 times for each examined temperature.

Phages stability at different pH

With certain adjustments, the *in vitro* testing of phage stability under various pH settings was carried out in accordance with the procedures (25). The stability of the three isolated phages was tested in CPG broth at pH levels 2, 5, 7, 9 and 11. After one h of incubation at 28 °C, the surviving phage particles were immediately counted using the double-layer agar plate method.

Phages stability to UV

The phage stability under UV radiation was tested with a modification (26). Petri dishes with 5 ml of high titer phage suspensions were individually positioned 50 cm from an ultraviolet light source (λ = 365 nm) and exposed for 10, 20, 30, 40, 50 and 60 min. As previously described, the double-layer agar plate method counted the phage particles that had survived after each exposure interval.

Effect of different agricultural chemicals on isolated phages

Potassium chloride (KCl), calcium chloride ($CaCl_2$), copper sulphate ($CuSO_4$) and copper chloride ($CuCl_2$) were chosen to be assessed on the isolated phages. Each salt was produced as stock solutions (100 mM). Each salt was diluted to a concentration of 0, 1, 2, 3, 4 and 0.5 mM. The phage solution with a final concentration of 108 pfu/ml and 1.5 ml of the host bacteria (Pcc DSM 30170 strain) were combined with 500 µl of each salt dilution. At room temperature, the mixture was incubated for 10 min. The double-layer method was used to calculate the number of plaques. A bacterial solution and phage were combined to create the control test without using the substances under investigation. The experiment was conducted in triplicates to minimize manual error.

Effect of different solvents on the isolated phages

The concentration effects of different solvents (chloroform, propanol, methanol and ethanol) on the isolated phages were studied with different concentrations, such as 30, 40, 50, 60 and 70% (v/v) for each selected solvent. Phages (10^{8} pfu/ml) in a 0.1M phosphate buffer solution (20 ml, pH7, supplemented with alcohol) were incubated at room temperature. After one h, the samples' plaque formation (units) was detected .

Statistical analysis

Data for phage stability to different temperature degrees, pH, UV radiation and different chemicals were analyzed by One-Way ANOVA using PASW STATICS 18 software. Duncan's Significant Difference test included in the software was used to compare means. Differences were considered statistically significant if $p < 0.05$.

Results

Isolation of bacteriophages

Three lytic phages with infection potential against Pcc DSM 30170 strain were isolated and designated as ϕPC1, ϕPC2 and ϕPC3. ϕPC1 was clear circular 3 mm plaque size. ϕPC2 was a clear circular 1 mm plaque size. ϕPC3 was a clear circular 2 mm plaque size (Fig. 1) (Table 1).

Transmission electron microscopy

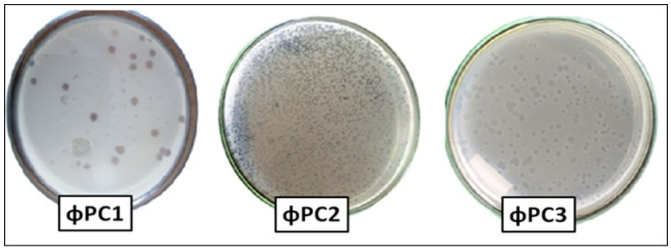

Fig. 1 Plaque Morphology of isolated phages

Table 1. Plaque morphology of the isolated phages

Plaque character	Phage		
	ϕPC1	ϕPC2	ϕPC3
Shape	Circle	Circle	Circle
Diameter in mm	3	1	2
Transparency	Clear	Clear	Clear
Presence of halo	Absent	Absent	Absent

The transmission electron microscope revealed that all the isolated phages belong to the Myoviridae family. ϕPC1 was to be approximately 284.6 nm in size, comprising the head (123.07 nm) and tail (161.53 nm). The phage ϕPC2 had an icosahedral head (114.28 nm) and tail (74.1 nm) with a visible base plate (11.4 nm) and tail fibers. The average length of the phage ϕPC2 from the top of the capsid to the base plate is 199.8 nm. The phage ϕPC3 was to be approximately 273.8 nm in size, comprising the head (118.4 nm) and tail (155.4) (Fig. 2) (Table 2).

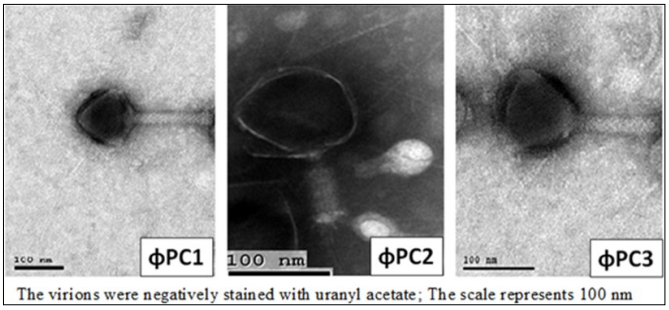

Fig. 2 Electron micrograph of isolated phage particles under TEM

Table 2. Morphological characters of the isolated phages as illustrated by transmission electron microscopic examination

phage	Head diameter	Tail length	Tail width	Base plates
ϕPC1	123.07	161.53	26.923	-
ϕPC2	114.28	74.1	31.42	11.42
ϕPC3	118.4	155.4	29.6	-

Restriction endonuclease analysis of phages DNA

All phages include dsDNA, as shown by the effective digestion of the DNA (Fig. 3). ϕPC1's genomic DNA was digested into 11 different-sized restriction bands, whereas ϕPC2 and ϕPC3's genomic DNA was digested into seventeen distinct restriction bands. The bacteriophage genome for ϕPC1, the presence of ϕPC2 and ϕPC3 is estimated to be 14, 20 and 22 kbp in length based on the total of the restriction fragment sizes.

Thermal stability of phages

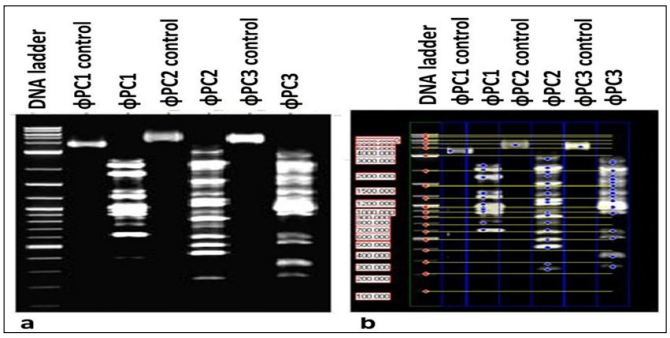

Fig. 3 Fragmentation pattern for phages with a mix of Hind III and Eco RI

The sensitivity of phages ϕPC1, ϕPC2 and ϕPC3 to various temperatures is represented in Fig. 4. The activity of the 3 phages remained stable in a range of temperatures up to 70 °C. The activity of Phages ϕPC2 and ϕPC3 was lost when subjected to a temperature of 80 °C for 10 min. ϕPC1was totally lost when subjected to 90 °C for 10 min. interestingly, ϕPC1, ϕPC2 and ϕPC3 phages survived at 50 °C with no significant loss in phage particle number.

Phages stability at different pH

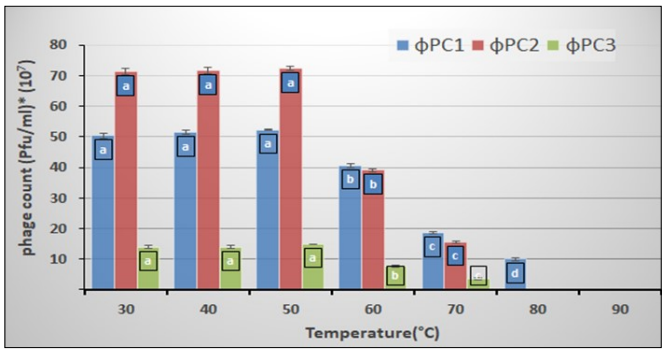

Fig.4 Phages stability at different temperatures

Fig. 5 illustrates how sensitive the phages ϕPC1, ϕPC2 and ϕPC3 are to different pH levels. When incubated at 28 °C, ϕPC1 and ϕPC2 maintained their capacity to lyse the Pcc DSM 30170 strain for up to 1 h at pH levels ranging from 3 to 9, with the highest stability at pH 6, 7 and 8. When incubated at 28 °C for an h, the activity of ϕPC3 remained stable for up to an h at pH levels ranging from 3 to 8 and it had the most significant stability at pH 6 and 7.

Phages stability to UV radiation

By increasing the exposure time from 0 to 60 min, UV radiation significantly reduced the number of plaques for each of ϕPC1, ϕPC2 and ϕPC3. ϕPC1, ϕPC2 and ϕPC3 retained their lytic capability after exposure to ultraviolet radiation for 60 min at 50 cm (Fig. 6).

Effect of different agricultural chemicals on the isolated phages

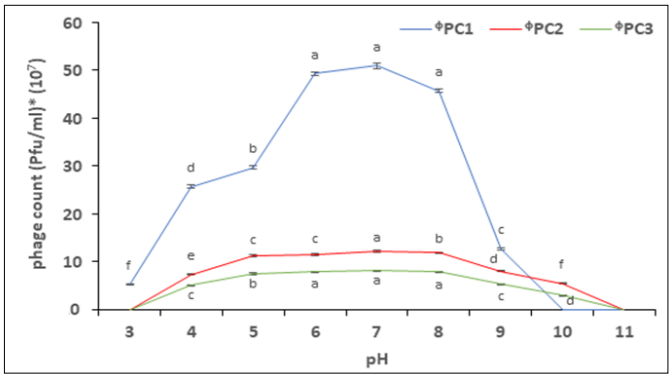

Fig. 5 Phages stability at different pH

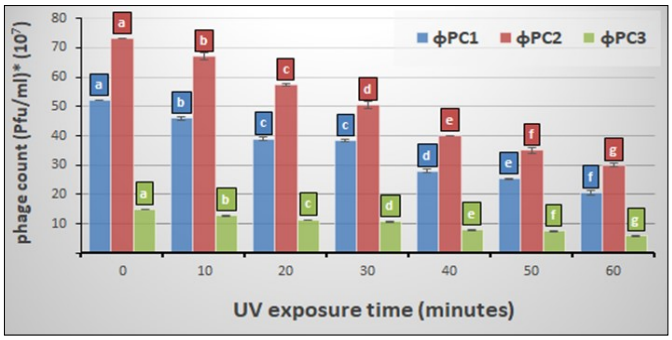

Fig. 6 Phages stability to UV

By increasing the chemical concentrations (from 0.1 to 0.5 mM), calcium chloride ($CaCl_2$) showed a significant increase in the activity of ϕPC1, ϕPC2 and ϕPC3, compared to the control. Potassium chloride (KCl) showed a significant increase in the activity of ϕPC1 and ϕPC2. Potassium chloride (KCl) increased the activity of ϕPC3; however, such an increase was insignificant. Copper sulphate ($CuSO_4$) and copper chloride ($CuCl_2$) showed a significant decrease in the activity of ϕPC3; however, such a decrease was insignificant for ϕPC1 and ϕPC2 (Table 3).

Effect of different solvents on the isolated phages

The only solvent tested that didn't cause the phage titer to decrease was chloroform. The count of the three isolated phages, ϕPC1, ϕPC2 and ϕPC3, was significantly reduced by increasing the solvent concentrations (from 30% v/v to 70% v/v) in propanol, ethanol and methanol when compared to the control. Following exposure to 60% propanol, 70% ethanol or 70% methanol, no ϕPC1 plaques were discovered. Following exposure to 50% propanol, 70% ethanol or 70% methanol, no ϕPC2 plaques were discovered. Following exposure to 50% propanol, 60% ethanol or methanol, no ϕPC3 plaques were discovered (Table 4).

Discussion

We successfully isolated three phages from sewage samples collected from the Sewage water treatment plant in Gabal Asfar, Khanka, Qalubeia, Egypt. to provide biological disease prevention strategies. It was not the first study to isolate bacteriophages lytic against *Pectobacterium carotovorum* as isolated bacteriophage PP16 from wastewater in the Moscow region using the highly virulent Pcc strain F002 (PB69) as a bacterial host.

DNA or RNA, either double- or single-stranded, makes up the genomes of phages. These genetic components are contained in a capsid, which can be polyhedral (Microviridae, Corticoviridae, Tectiviridae, Leviviridae and

Table 3. Effect of different agricultural chemicals on the isolated phages

Chemicals	Effect	Concentration (mM)	Phage count*(10^7 pfu/ml)		
			øPC1	øPC2	øPC3
$CaCl_2$	Increase phages activity ↑	0.1	18.00 ± 0.06^{e}	13 ± 0.17^{e}	15 ± 0.29^{e}
		0.2	20 ± 0.58^{d}	16 ± 0^{d}	17 ± 0.06^{d}
		0.3	25.00 ± 0^{c}	20 ± 0.58^{c}	18 ± 0.58^{c}
		0.4	28.00 ± 0.11^{b}	22 ± 0.35^{b}	19 ± 0.12^{b}
		0.5	30.00 ± 0.11^{a}	25 ± 0.29^{a}	20 ± 0.0^{a}
KCl	Increase phages activity ↑	0.1	11.20± 0.64^{d}	10 ± 0.46^{b}	10.2 ± 0.12^{c}
		0.2	13.00 ± 0.29^{c}	11 ± 0.23^{b}	10.5 ± 0.29^{bc}
		0.3	14.00 ± 0.35b^{c}	11.3 ± 0.75^{b}	12 ± 1.15^{ab}
		0.4	14.10 ± 0.06^{b}	13 ± 0.58^{a}	12.3 ± 0.40^{a}
		0.5	15.30 ± 0.17^{a}	13.3 ± 0.06^{a}	12.8 ± 0.12^{a}
$CuSO_4$	Decrease phages activity ↓	0.1	10.00 ±0.40^{a}	10 ± 0.58^{a}	9 ± 0.58^{a}
		0.2	9.70 ± 0.17^{a}	9.9 ± 0.06^{a}	8 ± 0.35^{ab}
		0.3	9.00 ± 0.17^{ab}	9.9 ± 0.11^{a}	7 ± 0.29^{bc}
		0.4	8.70 ± 0.75^{ab}	10 ± 0.0^{a}	7.2 ± 0.12^{bc}
		0.5	8.00 ± 0.58^{b}	9.8 ± 0.11^{a}	6 ± 0.58^{d}
$CuCl_2$	Decrease phages activity ↓	0.1	9.00 ± 0.06^{a}	10 ± 0.58^{a}	8.8 ± 0.12^{a}
		0.2	8.50 ± 0.29^{a}	9.5 ± 0^{a}	7.4± 0.23^{b}
		0.3	8.20 ± 0.17^{a}	9 ± 0.17^{a}	6.6 ± 0.69^{bc}
		0.4	7.00 ± 0.58^{b}	8 ± 0.17^{b}	6.4 ± 0.35^{bc}
		0.5	6.40 ± 0.06^{b}	7.5 ± 0.40^{b}	6 ± 0.35^{c}

Table 4. Effect of different solvents on the isolated phages

Solvents	Effect	Concentration % (v/v)	Phage count*(10^7 pfu/ml)		
			øPC1	øPC2	øPC3
Chloroform	No effect	30	9.83 ± 0.09^{b}	10.06 ± 0.09^{a}	10.03 ± 0.13^{a}
		40	9.93 ± 0.20^{ab}	10 ± 0.06^{a}	9.96 ± 0.09^{a}
		50	10.16 ± 0.03^{ab}	10.16 ± 0.03^{a}	10.10 ± 0.06^{a}
		60	10.23 ± 0.07^{a}	10.16 ± 0.09^{a}	10.10 ± 0.10^{a}
		70	10.06 ± 0.07^{ab}	10.06 ± 0.07^{a}	9.96 ± 0.14^{a}
Propanol	Decrease phages activity ↓	30	10.06 ± 0.07^{ab}	10.06 ± 0.07^{a}	9.96 ± 0.14^{a}
		40	5.3 ± 0.15^{a}	4.76 ± 0.14^{a}	2.94 ± 0.03^{a}
		50	3.06 ± 0.12^{b}	2.46 ± 0.26^{b}	1.01 ± 0.09^{b}
		60	0.16 ± 0.01^{c}	0.09 ± 0.00^{c}	0 ± 0^{c}
		70	0.00 ± 0.00^{c}	0 ± 0^{c}	0 ± 0^{c}
Methanol	Decrease phages activity ↓	30	9.8 ± 0.06^{a}	9.13 ± 0.07^{a}	9.9 ± 0.06^{a}
		40	9.36 ± 0.09^{b}	7.96 ± 0.09^{b}	9.66 ± 0.14^{b}
		50	7.9 ± 0.06^{c}	6.13 ± 0.14^{c}	6.06 ± 0.12^{c}
		60	2.23 ± 0.14^{d}	2.76± 0.12^{d}	0.07 ± 0.03^{d}
		70	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{d}
Ethanol	Decrease phages activity ↓	30	9.66 ± 0.09^{a}	9.9 ± 0.06^{a}	9.9 ± 0.15^{a}
		40	9.36 ± 0.09^{b}	9.16 ± 0.09^{b}	9.03 ± 0.03^{b}
		50	7.50 ± 0.29^{c}	7 ± 0.29^{c}	3.23 ± 0.18^{c}
		60	0.13 ± 0.03^{d}	0.25 ± 0.02^{d}	0 ± 0^{d}
		70	0.00 ± 0^{d}	0 ± 0^{d}	0 ± 0^{d}

Cystoviridae), filamentous (Inoviridae), pleomorphic (Plasmaviridae), or attached to a tail (Caudovirales). The Caudovirales order contains the Myoviridae family with a contractile tail and the Podoviridae family with a short tail, making up around 96% of the documented bacteriophages. The transmission electron microscope and genomic characteristics of the 3 isolated phages in this study revealed that they all belong to the Myoviridae family. ϕPC1 was to be approximately 284.6 nm in size, comprising head (123.07 nm) and tail (161.53 nm). The phage ϕPC2 had an icosahedral head (114.28 nm) and tail (74.1 nm) with a visible base plate (11.4 nm) and tail fibers. The average length of the phage ϕPC2 from the top of the capsid to the base plate is 199.8 nm. The phage ϕPC3 was to be approximately 273.8 nm in size, comprising of head (118.4 nm) and tail (55.4). Likewise, several *Myoviridae* bacteriophages infecting *Pcc* have been described: Pc1, Wc2, Wc3, Wc4 vB_PcaM -D1 and vB_PcaM-J3 infecting *Pcc*. All isolated phages include dsDNA, as shown by the effective digestion of the DNA. The genomic DNA of ϕPC1 digested in eleven restriction bands, whereas ϕPC2 and ϕPC3 digested in seventeen restriction bands of different sizes. The genome for ϕPC1, ϕPC2 and ϕPC3 is estimated to be 14, 20 and 22 kbp in length based on the total of the restriction fragment sizes. Their stability significantly influences agriculture's acceptance of biocontrol agents.

The isolated phages exhibited thermostability at elevated temperatures (up to 70 °C for ϕPC2 and ϕPC3 and 80 °C for ϕPC1). This is in accordance with, who suggested that bacteriophage PP1 1, with lytic activity against *Pcc,* was stable at relatively high temperatures. Similarly, found that Phage P7_Pc could maintain its viability against Pcc when stored at 45 °C and 55 °C for 1 h (19).

Phages were contagious in both acidic and alkaline settings, but excessive alkalinity completely suppressed them (pH 10 for ϕPC1, pH 11 for ϕPC2 and ϕPC3). The optimum infectivity of ϕPC1and ϕPC2 was obtained at pH range 6-8 and 6-7 for ϕPC3. This is in agreement with, who stated that the optimal pH for phages is neutral and the rise in acidity and alkalinity has an inverse relationship with the infectivity of the phage (36). As reported, the denaturation of phages' proteins is to blame for this.

ϕPC1, ϕPC2 and ϕPC3 retained their lytic capability after exposure to ultraviolet radiation for 60 min at 50 cm. Inversely, Myoviridae bacteriophages isolated could not survive 10 min of UV exposure, although 5 min of exposure cut their concentration in half as measured by a plaque assay (38).

Agricultural plants are frequently treated with different chemicals. To uncover potential interferences, we mixed phages with varying concentrations of some of the most commonly used agrochemicals and monitored the changes in phage titer after each treatment. By increasing the chemical concentrations (from 0.1 to 0.5 mM), calcium chloride ($CaCl_2$) and potassium chloride (KCl) showed a significant increase in the lytic activity of the 3 phages ϕPC1, ϕPC2 and ϕPC3. This rise in phage activity is likely due to higher rates of adsorption and penetration. Our

results are in accordance with, who showed that calcium and potassium ions increase the process of phage infectivity (39). Copper sulphate ($CuSO_4$) and copper chloride ($CuCl_2$) showed a significant decrease in the activity of ϕPC3; however, such a decrease was insignificant for ϕPC1 and ϕPC2. Despite its obvious efficacy, it is still unclear how copper inactivates phages. According to the majority of publications, redox cycling between the various copper species produces hydrogen peroxide (H_2O_2) and/or reactive oxygen species (ROS), which in turn causes phage inactivation. These molecules are known to damage DNA and proteins, the components of bacteriophages. Thus, avoiding using copper compounds in plots where phages are applied seems advisable.

Propanol, ethanol and methanol showed a significant decrease in the count of the 3 isolated phages, ϕPC1, ϕPC2 and ϕPC3, by increasing the concentrations (from 30 % v/v to 70 % v/v). ϕPC1, ϕPC2 and ϕPC3 were more tolerant to ethanol and methanol than propanol as 50-60% (v/v) propanol resulted in total loss in the phages count, but 60-70% (v/ v) ethanol, or methanol decreased the phages count to zero. Below these concentrations, the killing effect of alcohols against bacteriophages became much weaker. On the other hand, chloroform was the only one among the solvents tested that did not significantly reduce the phage titer. Other phages were found to tolerate chloroform, such as *Pectobacterium* phage CB7 isolated (42). After 1 h of exposure to chloroform, the phage was tolerable, with no discernible log-fold decrease in phage titer. In contrast, *Pectobacterium* phages isolated earlier were found to be sensitive to chloroform (43). 1 h of incubation with chloroform at room temperature caused a 100-fold titer drop.

Our findings offer significant information for developing a strategy to combat the *P. carotovorum* subsp. *carotovorum* caused bacterial soft rot disease.

Conclusion

Three phages lytic against *P. carotovorum* subsp. *carotovorum* were isolated, designated as (ϕPC1, ϕPC2, ϕPC3) and classified based on morphological and genomic characteristics as Myoviridae. Before applying phages to treat the soft rot disease caused by Pcc, we tested if the lytic activity of phages could be affected by some common chemicals and solvents generally used in agriculture. From these chemicals tested, calcium chloride and potassium chloride were found to increase the lytic activity of the phages and their adsorption on *Pcc.* On the other hand, copper sulphate, copper hydroxide and alcohols decreased the lytic activity of the phages against Pcc. Thus, it seems advisable to avoid using copper compounds and alcoholic solvents in plots where phages are applied.

Authors contributions

MHA performed the experiments and wrote the manuscript and participated in manuscript revising and editing. YAH conducted experimental methodology, participated in data analysis and representation and participated in manuscript revising and editing. NAY conceived and designed research, provided the used chemicals, provided practical guidance and participated in manuscript revising and editing. NMB provided the used chemicals, provided practical guidance and participated in manuscript revising and editing revising and editing. AA conceived and designed the research, conducted experimental methodology, participated in data analysis and representation. GD suggested the research point, investigated the article, conceived and designed the research, conducted experimental methodology. KA participated in manuscript revising, editing and publishing. All authors read and approved the article.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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