RESEARCH

Characterization of selected phages for biocontrol of food-spoilage pseudomonads

Daisuke Johno[1] · Yu Zhang[1,2] · Tahir Noor Mohammadi[1] · Junxin Zhao[1,3] · Yunzhi Lin[1] · Chen Wang[1] · Yuan Lu[1] · Marwa Nabil Sayed Abdelaziz[1] · Aye Thida Maung[1] · Chen-Yu Lin[1] · Mohamed El-Telbany[1] · Su Zar Chi Lwin[1] · Catherine Hofilena Damaso[1] · Yoshimitsu Masuda[1] · Ken-ichi Honjoh[1] · Takahisa Miyamoto[1]

Abstract

Pseudomonas spp., such as *P. fluorescens* group, *P. fragi*, and *P. putida*, are the major psychrophilic spoilage bacteria in the food industry. Bacteriophages (phages) are a promising tool for controlling food-spoilage and food-poisoning bacteria; however, there are few reports on phages effective on food-spoilage bacteria such as *Pseudomonas* spp. In this study, 12 *Pseudomonas* phages were isolated from chicken and soil samples. Based on the host range and lytic activity at 30 °C and 4 °C and various combinations of phages, phages vB_PflP-PCS4 and vB_PflP-PCW2 were selected to prepare phage cocktails to control *Pseudomonas* spp. The phage cocktail consisting of vB_PflP-PCS4 and vB_PflP-PCW2 showed the strongest lytic activity and retarded regrowth of *P. fluorescens* and *P. putida* at 30 °C, 8 °C, and 4 °C at a multiplicity of infection of 100. Nucleotide sequence analysis of the genomic DNA indicated that vB_PflP-PCS4 and vB_PflP-PCW2 phages were lytic phages of the Podoviridae family and lacked tRNA, toxin, or virulence genes. A novel endolysin gene was found in the genomic DNA of phage vB_PflP-PCS4. The results of this study suggest that the phage cocktail consisting of vB_PflP-PCS4 and vB_PflP-PCW2 is a promising tool for the biocontrol of psychrophilic food-spoilage pseudomonads during cold storage and distribution.

Keywords Psychrophilic *Pseudomonas* phage · Phage cocktail · Low temperature · Biocontrol · Optimization

Introduction

Pseudomonas spp. are aerobic, motile, and non-spore-forming bacilli widely found in the environment. In the food industry, *Pseudomonas* spp. are spoilage bacteria that cause problems in aerobically stored foods, particularly in

foods with high water content and neutral pH, such as red meat, fish, poultry, milk, dairy products, vegetables, and fruits (Raposo et al, 2017). In particular, the *P. fluorescens* group, along with the psychrophilic *P. fragi* and *P. putida*, is involved in the spoilage of milk, meat, and fish at low storage temperatures because they can grow aerobically under chilled conditions (Papadopoulou et al. 2020). Additionally, *Pseudomonas* spp. form strong biofilms, higher-order structures composed of polysaccharides, polypeptides, and extracellular nucleic acids (Scales et al. 2014; Fanelli et al. 2021). Bacterial cells in biofilms are more resistant to physical and chemical stresses than planktonic bacterial cells, making it difficult to achieve sufficient bactericidal effects under normal sterilization conditions (Simões et al. 2010).

Drug-resistant bacteria are a growing problem worldwide. Bacteriophages (phages) have attracted considerable attention as a promising solution (Thiel 2004). Phages are viruses that infect bacteria, and unlike antibiotics, which have a broad antibacterial spectrum, they have high host specificity. They are harmless to humans and can selectively

---

Daisuke Johno and Yu Zhang are the first authors and contributed equally to this work.

✉ Takahisa Miyamoto
tmiyamot@agr.kyushu-u.ac.jp

1   Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 744 Motooka, Nishi-Ku, Fukuoka 819-0395, Japan

2   State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

3   State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China

sterilize target host bacteria without affecting food characteristics (Sulakvelidze et al. 2001). These phages were shown to be effective against biofilms. Many phages, but not all, can produce polysaccharases or polysaccharide lyases and can access the biofilm interior through the water channel (Knecht et al. 2020; Sutherland et al. 2004). Therefore, it is useful in many fields, including clinical and food, as a novel antibacterial agent (Endersen and Coffey 2020). Although *Pseudomonas* phages have been known and studied for over half a century, studies have mainly focused on pathogenic bacteria (Ceyssens and Lavigne 2010) such as *P. aeruginosa* (Naknaen et al. 2023; Lister et al. 2009), *P. plecoglossicida* (Nishimori et al. 2000), and *P. syringae* (Hirano and Upper 2000). *Pseudomonas* spp. are most frequently observed in chilled food spoilage (Raposo et al, 2017), but few reports infecting such food spoilage *Pseudomonas* spp. (Tanaka et al. 2018; Lammens et al 2020).

In this study, we isolated *Pseudomonas* phages from food or soil samples using *Pseudomonas* spp. isolated from lettuce as hosts and investigated the various characteristics and the combined use of the phages to determine the optimal combination as a phage cocktail and examined the potential use of the phage cocktail as a non-thermal sterilization method, mainly in the food industry.

Materials and methods

Bacterial strains and culture media

Pseudomonas fluorescens groups No. 257 and No. 271 were isolated from lettuce leaves in our laboratory and identified as the *P. fluorescens* group (*P. fluorescens group*, *P. fluorescens*, or *P. reactans*) using the ID test NF-18 (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and 16S rRNA sequencing using primers reported by Lane (1991). *P. aeruginosa* NBRC13275, *P. alcaligenes* NBRC14159, *P. fluorescens* NBRC14160, *P. fragi* NBRC3458, *P. oleovorans* NBRC13583, *P. putida* NBRC14164, and *P. tolaasii* NBRC15100 were obtained from the Biological Resource Center (NBRC). Bacterial strains grown on tryptic soy agar (TSA; Becton, Dickinson and Company, Sparks, MD, USA) were stored at 4 °C until use.

Isolation, purification, and propagation of Pseudomonas phages

The *Pseudomonas fluorescens* group No. 257 strain was used as the bacterial host for phage isolation. Eighty-five food or soil samples were used for phage isolation. Food samples were obtained from 65 chickens, three beefs, six porks, one fish, and two vegetables purchased from various supermarkets in Fukuoka City, Japan. Eight soil samples were

obtained from the Fruit Tree Research Institute of the Fukushima Prefectural Agricultural Research Center (Iizaka-cho, Fukushima City, Fukushima Prefecture, Japan). Each sample (50 g) was aseptically placed in a sterile stomacher bag and homogenized in 100 mL of tryptic soy broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) with 10 mM $CaCl_2$ for 1 min. The homogenate was inoculated with 100 μL of the host culture and incubated at 30 °C for 24 h. Then, 10 mL of the culture was withdrawn and centrifuged at $12,000 \times g$ at 4 °C for 20 min. The supernatants were filtered through a 0.45-μm pore size sterile membrane filter (Merck Millipore, Ireland). Filtrates were mixed at a 1:1 ratio with the host cell suspension and incubated for 1 h at 30 °C. After incubation, 200 μL of the mixture was added to 4 mL of molten top agar (TSB containing 0.5% (w/v) agar) at 55 °C, poured onto plates of TSA, and incubated at 30 °C for 24 h for plaque formation. An observed plaque was picked up, suspended in 1 mL of saline magnesium (SM) buffer (0.05 M Tris–HCl buffer, pH 7.5, containing 0.1 M NaCl, 0.008 M $MgSO_4$, and 0.01% gelatin) and tenfold serially diluted with the same buffer. The phages were purified as follows. The phage dilution (100 μL) and 100 μL of the host culture were mixed and incubated at 30 °C for 1 h. The mixture was then added to 4 mL top agar at 55 °C, immediately poured onto TSA plates, and incubated overnight at 30 °C for plaque formation. This purification procedure was repeated thrice. Purified phages with high titers ($> 10^8$ PFU/mL) were stored at 4 °C until use. The phages were named to include information about phage morphology and host according to the proposal by Kropinski et al. (2009).

Host range determination

The host range of the purified phages was assessed using spot tests on the nine *Pseudomonas* strains listed in Fig. 1. The phage suspension (10 μL) was spotted onto molten top agar inoculated with each host bacteria and incubated at 30 °C for 24 h. Plaque formation was interpreted as lysis of the bacterial host, and no plaque was interpreted as no lysis.

Lytic activity test

To assess the effects of the isolated *Pseudomonas* phages on planktonic cells, *P. fluorescens* group No. 271 precultured at 30 °C in TSB was diluted with TSB to attain a cell concentration of approximately 10^7 CFU/mL. The cell suspension was inoculated with a phage suspension (10^7 PFU/mL) at a multiplicity of infection (MOI) of 1. For the control, the same volume of SM buffer was used instead of the phage suspension. The mixture was incubated at 30 °C for 24 h with shaking at 130 rpm. At 0, 4, 8, and 24 h of incubation, the viable counts were measured using the plating method using TSA plates after overnight incubation at 30 °C. The
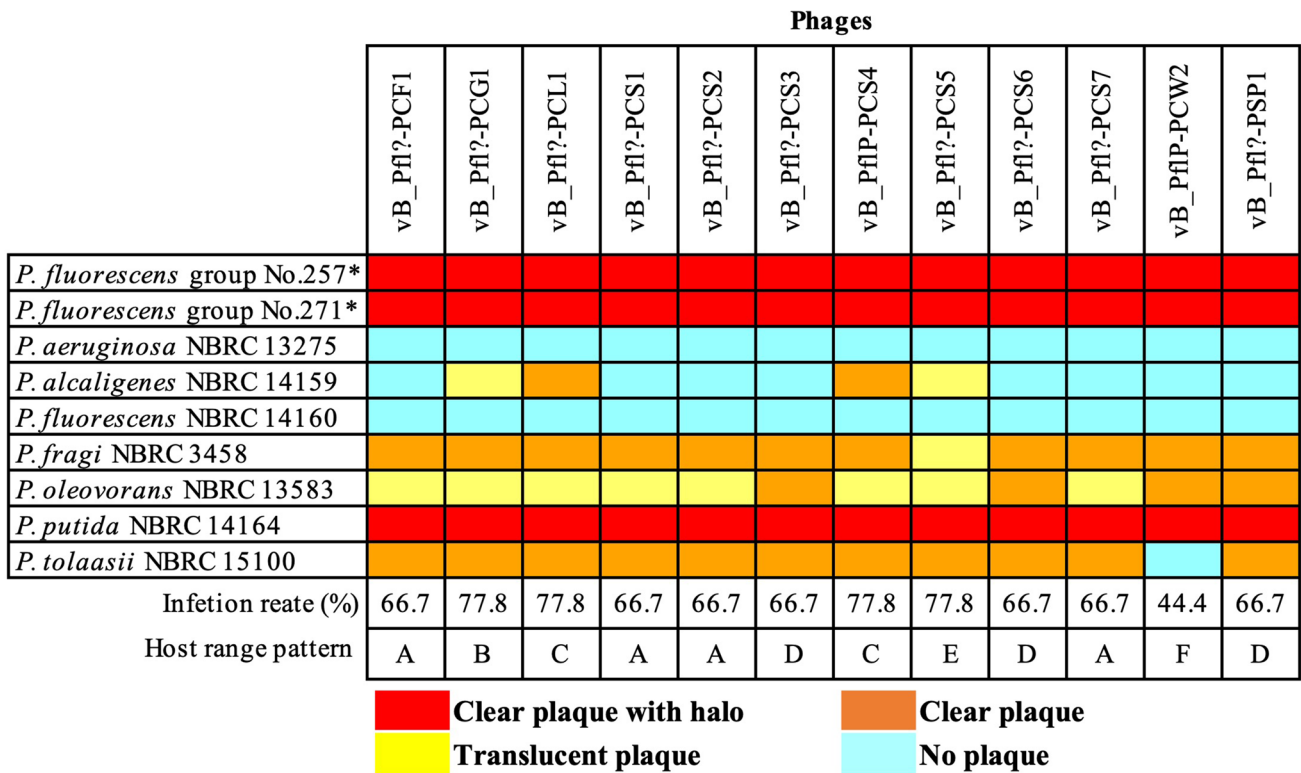
Phages

	vB_Pfl?-PCF1	vB_Pfl?-PCG1	vB_Pfl?-PCL1	vB_Pfl?-PCS1	vB_Pfl?-PCS2	vB_Pfl?-PCS3	vB_PflP-PCS4	vB_Pfl?-PCS5	vB_Pfl?-PCS6	vB_Pfl?-PCS7	vB_PflP-PCW2	vB_Pfl?-PSP1
P. fluorescens group No.257*	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo
P. fluorescens group No.271*	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo
P. aeruginosa NBRC 13275	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque
P. alcaligenes NBRC 14159	No plaque	Translucent plaque	Clear plaque	No plaque	No plaque	No plaque	Clear plaque	Translucent plaque	No plaque	No plaque	No plaque	No plaque
P. fluorescens NBRC 14160	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque	No plaque
P. fragi NBRC 3458	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Translucent plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque
P. oleovorans NBRC 13583	Translucent plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Translucent plaque	Clear plaque
P. putida NBRC 14164	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo	Clear plaque with halo
P. tolaasii NBRC 15100	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	Clear plaque	No plaque	Clear plaque
Infection reate (%)	66.7	77.8	77.8	66.7	66.7	66.7	77.8	77.8	66.7	66.7	44.4	66.7
Host range pattern	A	B	C	A	A	D	C	E	D	A	F	D

Legend: ■ Clear plaque with halo (red) ■ Clear plaque (orange) ■ Translucent plaque (yellow) ■ No plaque (cyan)

Fig. 1 Heat map for host range of the phages against various *Pseudomonas* strains. Source of isolations was PCF1 from chicken fallopian tube, PCG1 from chicken gizzard; PCL1 from chicken liver; PCS1-7 from chicken skin, PCW2 from chicken wings; and PSP1 from soil. *Isolated from lettuce leaf

lytic activity tests were performed at 4 °C. The host cell suspension (10^7 CFU/mL) was inoculated with a phage suspension (10^9 PFU/mL) at an MOI of 100. The mixture was incubated at 4 °C for 96 h, with agitation at 130 rpm. Viable cell counts were measured by plating the assays on TSA. Colonies were counted after overnight cultivation at 4 °C followed by overnight cultivation at 30 °C.

To assess the effects of phage cocktails, equal volumes of different phage suspensions with 10^7 PFU/mL were mixed to prepare a phage cocktail (10^7 PFU/mL). A host cell suspension (10^7 CFU/mL) in TSB was inoculated with the phage cocktail at an MOI of 1. The mixture was incubated at 30 °C for 96 h, with agitation at 130 rpm. An aliquot of the mixture was withdrawn at a suitable interval, and viable counts were measured by the plating assay using TSA after overnight cultivation at 30 °C.

The combined effects of phages vB_PflP-PCS4 and/or vB_PflP-PCW2 on the viability of *P. fluorescens* group No. 271 and *P. putida* NBRC14164 were determined at 30 °C, 8 °C, and 4 °C. A bacterial cell suspension (10^7 CFU/mL) in TSB was inoculated with a single phage (10^9 PFU/mL) or a phage cocktail (10^9 PFU/mL) at an MOI of 100. An equal volume of SM buffer was used instead of the phage suspension used as a control. The mixtures were incubated at 30 °C, 8 °C, and 4 °C for 168 h with agitation at 130 rpm. Viable counts were measured by plating the cells on TSA. Colonies were counted after overnight incubation at 30 °C. In the case of the measurement of viable counts of bacteria treated with phages at 8 °C and 4 °C, plates were first incubated overnight at 8 °C and 4 °C, respectively, before overnight incubation at 30 °C.

Determination of latent period and burst size of phages

One-step growth curve experiments were performed according to the methods described by Son et al. (2018) and Masuda et al. (2021) with some modifications. The host strain *P. fluorescens* group No. 271 in the stationary phase of growth and the vB_PflP-PCS4 or vB_PflP-PCW2 phage solutions were mixed at final concentrations of 10^8 CFU/mL and 10^6 PFU/mL in 500 mL of TSB (MOI of 0.01) and incubated at 30 °C for 5 min. Mixed solutions were centrifuged at 4 °C and $12,000 \times g$ for 3 min, and the supernatant was discarded to remove excess phage particles. The pellets were resuspended in 10 mL of prewarmed TSB at 30 °C, and suspensions were incubated at 30 °C for 40 min. Every 5 min from the start of incubation, 200 mL of suspension was

transferred into a 1.5-mL Eppendorf tube and centrifuged at 4 °C and $12,000 \times g$ for 3 min. After centrifugation, supernatants were transferred into new tubes and kept at 4 °C as phage solutions until they were used as templates for phage quantification using quantitative PCR (qPCR). The burst size of each phage was calculated by dividing the phage particle number at the end of a single infectious cycle by the phage particle number at time zero, using data from three independent experiments.

In this study, the number of phage particles in each phage solution was quantified using a plaque-forming assay and qPCR with each phage solution as a template, as previously described (Peng et al. 2018; Masuda et al. 2021). qPCR was performed using the Mx3000P real-time PCR system (Stratagene, La Jolla, CA, USA) and Thunderbird SYBR qPCR master mix (TOYOBO Co., Ltd., Osaka, Japan) according to the manufacturer's instructions. Phage solutions were first quantified using a plaque-forming assay to prepare standard solutions (10^4–10^7 PFU/mL). Standard curves for both vB_PflP-PCS4 and vB_PflP-PCW2 phages were prepared using these phage solutions as templates and the primers PCS4-11629Fw/PCS4-11733Rv and PCW-15735Fw/PCW2-15836Rv (Table S1).

pH and thermal stability test

The effect of pH on phage activity was determined at 25 °C. The pH of the SM buffer was adjusted with 0.1 M HCl and 0.1 M NaOH at pH values ranging from 3 to 12. Then, 20 μL of phage suspension (1×10^7 PFU/mL for vB_PflP-PCS4 and 1×10^6 PFU/mL for vB_PflP-PCW2) was added to 380 μL aliquots of SM buffer with different pH values and incubated at 25 °C for 24 h. For the thermal stability test, 200 μL of the same phage suspensions was incubated at 4 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C for 1 h. After pH and thermal treatments, the number of surviving phages was determined using a plaque-forming assay.

Genetic analysis

Nucleic acids were extracted from newly purified phages vB_PflP-PCS4 and vB_PflP-PCW2 suspensions (10^9 PFU/mL). The phages were lysed using proteinase K at 70 °C for 10 min. After lysis, DNA was purified using a Phage DNA Isolation Kit (NORGEN, Cat. 46800, 46850 ON, Canada). The genomic DNA was sequenced using an Illumina HiSeq system (HiSeq-PE150). The read sequences were assembled de novo using the VelvetOptimizer version 1.2.10 (Zerbino and Birney 2008). The assembly gaps were closed using GMcloser (Kosugi et al. 2015). The assembled genome was annotated using the Prokka 1.14.6 software (Seemann 2014). Open reading frames (ORFs) were determined using the NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffi

nder/). The function of each ORF was predicted by homology searching using NCBI BLASTP (Ramsay et al. 2000). The complete genome sequences of phages vB_PflP-PCS4 and vB_PflP-PCW2 have been deposited in the GenBank database under accession numbers OK094519.1 and OM471789, respectively. The absence of tRNA, virulence, and toxic genes was confirmed using tRNAscan-SE 2.0 (Chan et al. 2021; Lowe and Chan 2016) and Virulence-Finder 2.0 (Clausen et al. 2018; Joensen et al. 2014; Malberg Tetzschner et al. 2020).

Statistical analysis

Data points from all experiments represent the mean of results from at least three independent experiments, and error bars indicate standard deviations. All data were subjected to Student's t-test to ensure statistically significant differences at $P < 0.05$.

Results

Isolation and purification of Pseudomonas phages from meat and soil samples

In the current study, 85 food and soil samples were tested, and 12 lytic phages were isolated using a lettuce-derived *P. fluorescens* group strain as a host. Among them, seven phages were isolated from chicken skin, one from chicken wing, one from chicken gizzard, one from chicken liver, one from chicken fallopian tubes, and one from soil.

Host range of isolated phages

The lytic activities of the isolated phages against *Pseudomonas* spp. are listed in Fig. 1. All the phages were active against lettuce-derived *P. fluorescens* strains and *P. putida* NBRC14164. Some phages were active against *P. alcaligenes* NBRC14159, *P. fragi* NBRC3458, *P. oleovorans* NBRC13583, and *P. tolaasii* NBRC15100. None of the phages were active against *P. aeruginosa* NBRC13275 and *P. fluorescens* NBRC14160. The phages were grouped into six groups according to their host range patterns.

Phages vB_PflP-PCS4, vB_Pfl?-PCS1–3, and vB_Pfl?-5–7 were isolated from different chicken skin samples. Among them, vB_Pfl?-PCS1, 2, and 7 showed the same host range pattern A, and vB_Pfl?-PCS3 and vB_Pfl?-PCS6 showed the same host range pattern D. Since the phages were isolated from the different chicken samples purchased at the different supermarket at different day, it seems that they were different phages. Genomic DNA sequencing is required to confirm that these phages showing the same host range pattern are different phages.

Lytic activity of purified phages at 30 °C and 4 °C

The lytic activity of the 12 phages was determined at different temperatures. The lytic activity of the phages at 30 and 4 °C is shown in Fig. 2. At 30 °C, phages vB_Pfl?-PCS2, vB_Pfl?-PCS3, vB_Pfl?-PCS5, vB_Pfl?-PCS6, vB_PflP-PCW2, and vB_Pfl?-PSP1 largely decreased the viable counts of the lettuce-derived *P. fluorescens* group No. 271 strain by 3 to 4 log at 4 or 8 h of incubation. At 4 °C, the phages vB_Pfl?-PCL1 and vB_Pfl?-PSP1 decreased the viable count by approximately 2.5 log after 48 and 24 h of incubation, respectively.
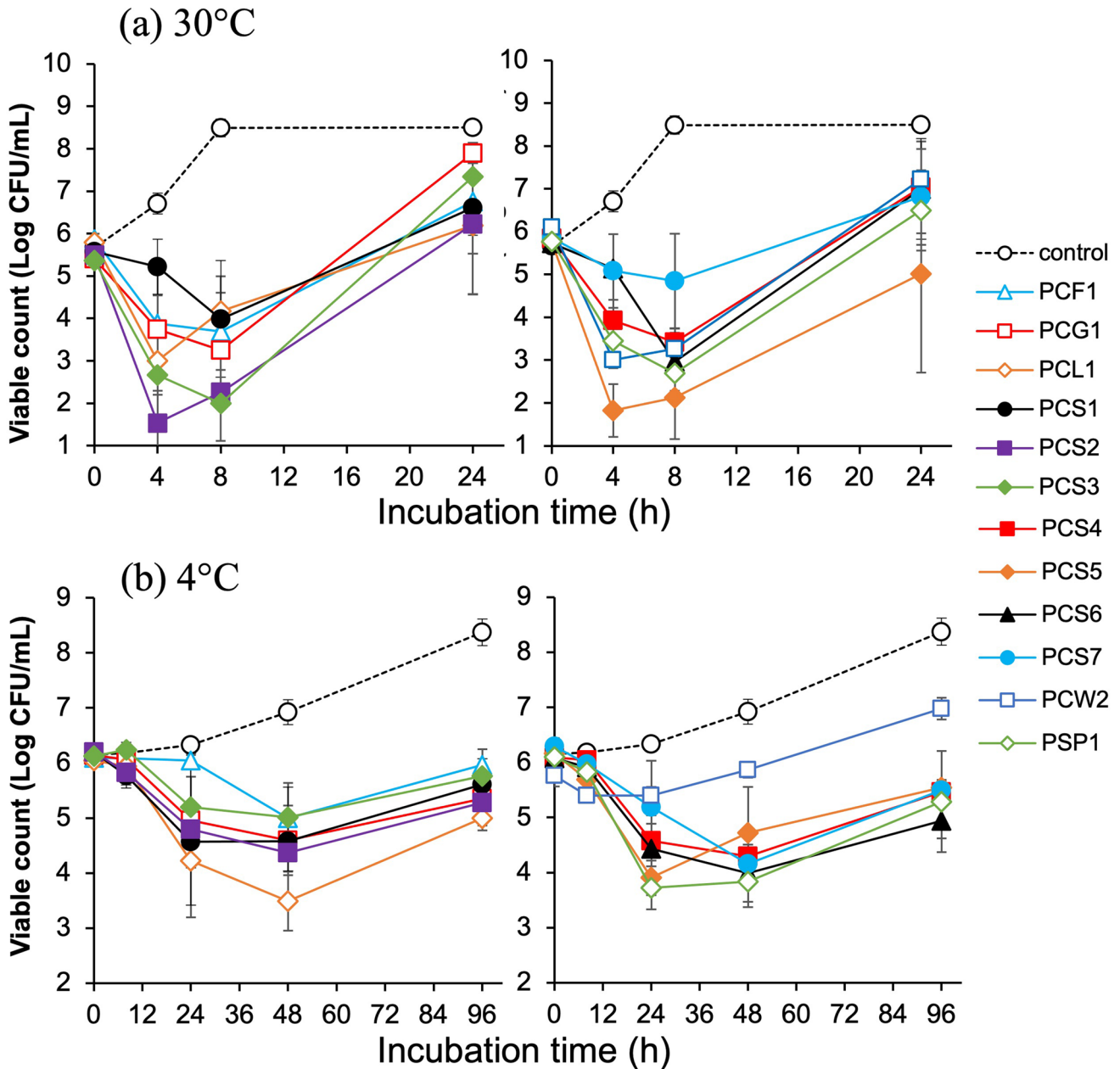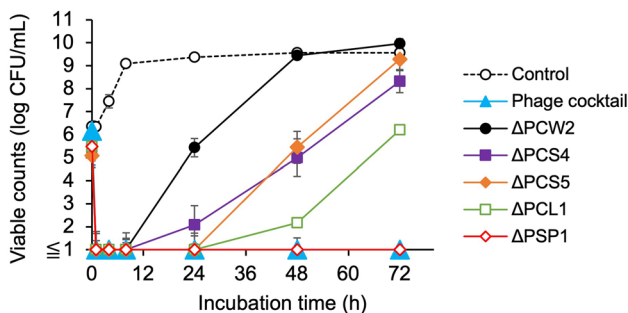

Fig. 2 Effects of phages on viability of *P. fluorescens* group No. 271 at 30 °C and 4 °C. *P. fluorescens* group No. 271 suspension in TSB was inoculated with phage suspension at an MOI of 1 and 100 at 30 °C and 4 °C, respectively. For the control, an SM buffer was used. The mixtures were incubated at 30 °C and 4 °C with shaking, and viable counts were determined. Symbols: ○, control without phage; △, vB_Pfl?-PCF1; □, vB_Pfl?-PCG1; ◇, vB_Pfl?-PCL1; ●, vB_Pfl?-PCS1; ■, vB_Pfl?-PCS2; ◆, vB_Pfl?-PCS3; ■, vB_PflP-PCS4; ◆, vB_Pfl?-PCS5; ▲, vB_Pfl?-PCS6; ●, vB_Pfl?-PCS7; □, vB_PflP-PCW2; ◇, vB_Pfl?-PSP1

Lytic activity of phage cocktails at 30 °C

Based on the host range patterns and lytic activities at different temperatures, vB_Pfl?-PCL1 (pattern C), vB_PflP-PCS4 (pattern C), vB_Pfl?-PCS5 (pattern E), vB_PflP-PCW2 (pattern F), and vB_Pfl?-PSP1 (pattern D) were selected to test the combined effects of the phages. Among the two phages (pattern C) with the widest host range, vB_Pfl?-PCL1 and vB_PflP-PCS4 were selected because of the strongest lytic activity at 4 °C and strong lytic activity both at 30 °C and 4 °C, respectively. Among three phages (pattern D), vB_Pfl?-PSP1 was selected since it showed relatively strong lytic activity both at 30 °C and 4 °C than those of the other two phages. Phage vB_Pfl?-PCS5 was selected for cocktail testing, because the phage (pattern E) showed unique and the widest host range different from those of pattern C phages. Phage vB_PflP-PCW2 (pattern F) was selected for cocktail testing, because the phage showed a unique host range. In contrast, phage vB_Pfl?-PCS2 showed very strong lytic activity at 30 °C among the four phages (pattern A) showing the second widest host range. The host range was covered by the phages with patterns C and D. Although phage vB_Pfl?-PCG1 (pattern B) showed unique and the widest host range like patterns C and E, the lytic activity was not strong both at 30 °C and 4 °C.

Four phage cocktails were prepared by removing one phage from the 5-phage cocktail. The phage cocktails were tested for lytic activity against the *P. fluorescens* group No. 271 strain. The effects of 4- and 5-phage cocktails on the viability of the *P. fluorescens* group No. 271 strain at MOIs of 1 and 30 °C in TSB are shown in Fig. 3. All cocktails decreased the viable counts to below the lower limit of

detection at 1 h. The viable count was less than the lower limit of detection for 8 h in all cocktail treatments. Viable cell counts did not increase 72 h after treatment with the 5-phage cocktail and the phage cocktail without vB_Pfl?-PSP1. The viable cell count increased after 48 h in the presence of phages without vB_Pfl?-PCL1. Viable counts increased to the same level as those of the control in the presence of phage cocktails without vB_PflP-PCS4, PCS5, or vB_PflP-PCW2. These results suggest that vB_Pfl?-PSP1 and vB_Pfl?-PCL1 have little or no effect on suppressing the growth of resistant bacteria in combination with other phages.

According to the results, a phage cocktail with three phages, vB_PflP-PCW2, vB_PflP-PCS4, and PCS5 and 2-phage cocktails consisting of two of the three phages were tested. The effects of 2- and 3-phage cocktails on the viability of *P. fluorescens* group No. 271 at MOIs of 1 and 30 °C in TSB are shown in Fig. 4. In all the cells treated with the phage cocktails, the viable counts of *P. fluorescens* decreased to less than the lower limit of detection at 1 h and did not increase for 8 h from the start of incubation. However, the count increased to > 6 log CFU/mL after treatment with the phage cocktail without vB_PflP-PCW2. The viable count was the lowest after treatment with the phage cocktail without PCS5. These results indicate that the phage cocktail consisting of phages vB_PflP-PCS4 and vB_PflP-PCW2 showed the highest performance against *P. fluorescens* group No. 271 among the phage cocktails tested. These
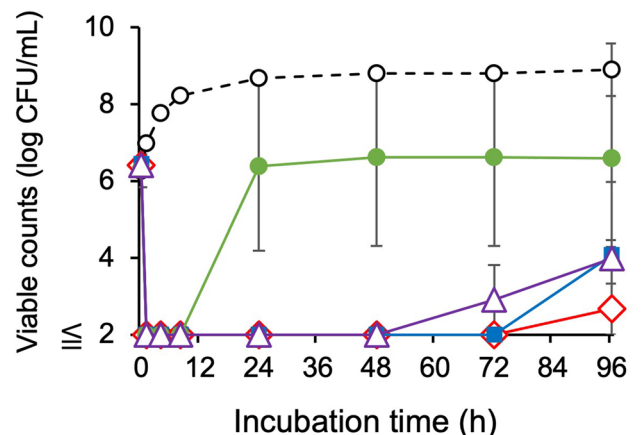

Fig. 3 Effects of 4- and 5-phage cocktails on the viability of *P. fluorescens* group No. 271 at 30 °C. *P. fluorescens* group No. 271 suspension in TSB was inoculated with phage cocktails at an MOI of 1. For the control, an SM buffer was used. Four-phage cocktails were prepared by removing one phage from a 5-phage cocktail (vB_Pfl?-PCL1 + vB_PflP-PCS4 + vB_Pfl?-PCS5 + vB_PflP-PCW2 + vB_Pfl?-PSP1). The mixtures were incubated at 4 °C with shaking, and viable counts were determined. Symbols: ○, control without phages; ▲, 5-phage cocktail; ●, cocktail without vB_PflP-PCW2; ■, cocktail without vB_PflP-PCS4; ◆, cocktail without vB_Pfl?-PCS5; □, cocktail without vB_Pfl?-PCL1; ◇, cocktail without vB_Pfl?-PSP1

Fig. 4 Effects of 2- and 3-phage cocktails on the viability of *P. fluorescens* group No. 271 at 30 °C. *P. fluorescens* group No. 271 suspension in TSB was inoculated with phage cocktails at an MOI of 1. For the control, an SM buffer was used. Two-phage cocktails were prepared by removing one phage from a 3-phage cocktail (vB_PflP-PCS4 + vB_PflP-PCW2 + vB_Pfl?-PSP1). The mixtures were incubated at 30 °C with shaking, and viable counts were determined. Symbols: ○, control without phages; △, 3-phage cocktail; ●, cocktail without vB_PflP-PCW2; ■, cocktail without vB_PflP-PCS4; ◇, cocktail without vB_Pfl?-PSP1

two phages were selected for characterization and genomic DNA sequencing.

Characterization of phages vB_PflP-PCS4 and vB_PflP-PCW2

Phages vB_PflP-PCS4 and vB_PflP-PCW2 formed plaques with large and small halos, respectively (Fig. 5a, b). A one-step growth experiment determined the latent time and burst size of phages vB_PflP-PCS4 and vB_PflP-PCW2. As shown in Fig. S1, phage vB_PflP-PCS4 had a latent time

Fig. 5 Characteristics of phages vB_PflP-PCS4 and vB_PflP-PCW2. Plaque morphology of phages **a** vB_PflP-PCS4 and **b** vB_PflP-PCW2 on the host *P. fluorescens* group No. 271 strain. Phages vB_PflP-PCS4 (○) and vB_PflP-PCW2 (△) were incubated in modified TSB broth at **c** different temperatures for 1 h and **d** different pH values at 25 °C for 24 h. The experiments were performed in triplicate, and error bars indicate standard errors of the mean. Bar: 10 mm

of 10 min and a burst size of 15 ± 2.0 PFU/bacterial cell. Phage vB_PflP-PCW2 had a latent time of 15 min and a burst size of 12 ± 0.3 PFU/bacterial cell. Both phages had small burst sizes; however, vB_PflP-PCS4 had a short lytic cycle. According to the results of the thermal stability test (Fig. 5c), the titers of phages vB_PflP-PCS4 and vB_PflP-PCW2 did not change after heating at 50 °C for 1 h but decreased gradually with increasing heating temperature from 60 to 80 °C (Fig. 5c). The effect of pH on the titer of the phages is shown in Fig. 5d. The phages were stable for 24 h at pH values ranging from 3 to 11. Phage vB_PflP-PCW4 was stable at pH 2.

The genome maps of phages vB_PflP-PCS4 and vB_PflP-PCW2 are shown in Fig. 6. Phage vB_PflP-PCS4 had a circular double-stranded DNA genome composed of 39,191 bp, with an overall G + C content of 58% (Fig. 6a). In the vB_PflP-PCS4 genome, 46 ORFs were identified; however, no tRNA, virulence, or toxic genes were identified using tRNAscan-SE 2.0 and VirulenceFinder2.0. A BLAST search indicated that the vB_PflP-PCS4 phage genome had 97.8% similarity to the *P. fluorescens* phage UNO-SLW1 complete genome (accession code NC_047873.1). Among these, 26 ORFs (56.5%) were assigned known functions, and 20 (43.5%) were assigned hypothetical functions, including one unmatched hypothetical protein. The results of the genome annotation are presented in Table S2. These proteins were categorized into four groups: (i) phage structure and packaging (connector protein, major capsid protein, tail spike protein, DNA encapsidation protein, collar protein, and tail protein); (ii) DNA replication (DNA polymerase, RNA polymerase, exonuclease, and primase/helicase); (iii) host lysis (N-acetylmuramoyl-L-alanine amidase, holin, and RZ lysis protein); and (iv) hypothetical protein. Phage vB_PflP-PCS4 had an N-acetylmuramoyl-L-alanine amidase endolysin gene (gpp19). The highest identity was found for the gene encoding lysin of *P. fluorescens* phage UNO-SLW1 (accession code YP_009793708) with 87.58% identity. Therefore, gpp19 in vB_PflP-PCS4 appears to encode a novel endolysin.

As shown in Fig. 6b, phage vB_PflP-PCW2 had a circular double-stranded DNA genome composed of 40,117 bp, with an overall G + C content of 59%. In the vB_PflP-PCW2 genome, 48 ORFs were identified; however, no tRNA, virulence, or toxicity genes were identified. A BLAST search indicated that the vB_PflP-PCW2 phage genome had 98.04% similarity with the *P. syringae* pv. Actinidiae phage CHF7 complete genome (accession code MN729596.1). The results of the genome annotation are listed in Table S3. Among them, 24 ORFs (50%) were assigned known functions, and 24 (50%) were assigned hypothetical functions, including one unmatched hypothetical protein. Phage vB_PflP-PCW2 also contained an N-acetylmuramoyl-L-alanine amidase endolysin gene (gpp27); however, the nucleotide
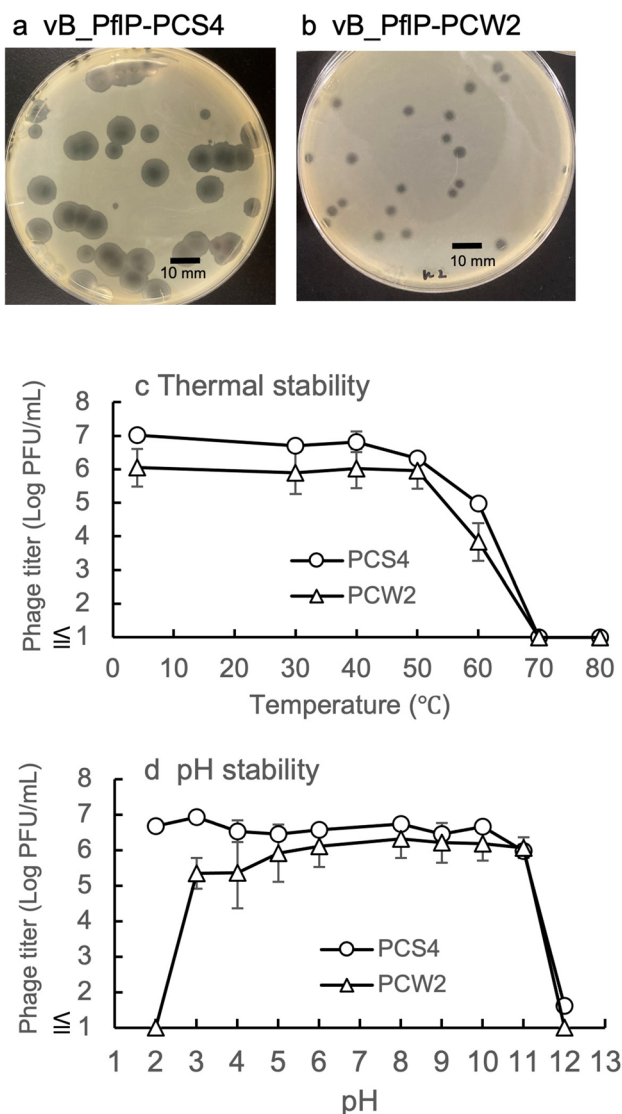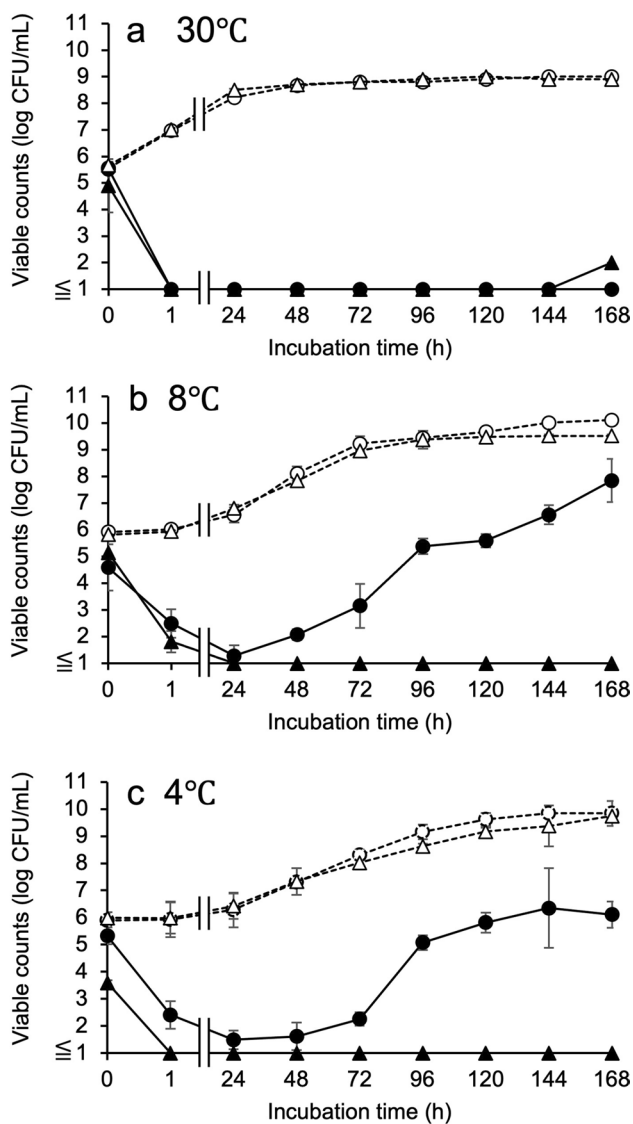
Fig. 6 Genome maps of phage **a** vB_PflP-PCS4 and **b** vB_PflP-PCW2. Arrows: green, phage structure and packaging; orange, DNA replication; yellow, host lysis; red–purple, hypothetical protein. The accession numbers of vB_PflP-PCS4 and vB_PflP-PCW2 are OK094519.1 and OM471789, respectively

a vB_PflP-PCS4

b vB_PflP-PCW2

sequence of this gene showed 100% similarity to that of the lysozyme of *Pseudomonas* phage CHF19 (accession code QHB47983.1). *P. fluorescens* phage UNO-SLW1 (Lu et al. 2017) and *P. syringae* pv. Actinidiae phage CHF7 (Flores et al. 2020) were reported to be the Podoviridae family. According to the high identity in the nucleotide sequences of the genomic DNAs obetweenvB_PflP-PCS4 and *P. fluorescens* phage UNO-SLW1 and vB_PflP-PCW2 and *P. syringae* pv. Actinidiae phage CHF7, these phages seem to belong to the Podoviridae family.

Effects of phage cocktail with phages vB_PflP-PCS4 and vB_PflP-PCW2 on viability of P. fluorescens group No. 271 and P. putida NBRC14164 in broth at 30 °C, 8 °C, and 4 °C

The effects of a phage cocktail with phages vB_PflP-PCS4 and vB_PflP-PCW2 at an MOI of 100 were determined on the viability of lettuce-derived *P. fluorescens* group No. 271 and *P. putida* NBRC14164 strains at 30 °C, 8 °C, and 4 °C (Fig. 7). At 30 °C, the viable count of *P. fluorescens* group

Fig. 7 Effects of phage cocktail composed of vB_PflP-PCS4 and vB_PflP-PCW2 on the viability of *Pseudomonas* strains at different temperatures. *P. fluorescens* group No. 271 (○,●) and *P. putida* NBRC14164 (△,▲) were incubated in the absence (open symbols) and presence (closed symbols) of the phage cocktail (vB_PflP-PCS4 and vB_PflP-PCW2) at an MOI of 100

No. 271 decreased to the lower limit of detection (less than 1 log CFU/mL) after 1 h, and the bacterial regrowth was inhibited for 144 h (Fig. 7). At 8 °C and 4 °C, against *P. fluorescens* group No. 271, the phage cocktail did not completely inhibit the regrowth of *P. fluorescens*, but the viable counts were lower by 2.2 and 3.5 log, respectively, compared with those of the control at 168 h (Fig. 7). In contrast, at all temperatures, the viable count of *P. putida* NBRC14164 decreased to the lower limit of detection after 1 h, and bacterial regrowth was inhibited after 168 h in the presence of the phage cocktail (Fig. 7). Therefore, the combined use of phages vB_PflP-PCS4 and vB_PflP-PCW2 was more effective against *P. putida* NBRC14164 than against lettuce-derived *Pseudomonas* spp. No.271 at 30 °C, 8 °C, and 4 °C.

Discussion

Food spoilage is a serious global problem in developed and developing countries. *Pseudomonas* spp. produce many thermotolerant lipolytic and proteolytic enzymes, reducing the quality and shelf life of raw and processed foods. Food spoilage caused by *Pseudomonas* spp. occurs in milk, dairy products, meat, fish, water, fruits, and vegetables (Papadopoulou et al. 2020; Singh, 2017). In this study, we isolated and characterized phages against food-spoilage pseudomonads for the biocontrol of bacteria in foods during cold storage. Twelve *Pseudomonas* phages were isolated from a soil sample and different chicken samples obtained on different days at different supermarkets and were grouped into six groups according to their host range patterns. Phages vB_PflP-PCS4, vB_Pfl?-PCS1–3, and vB_Pfl?-PCS5–7 were isolated from different chicken skin samples. Among them, vB_Pfl?-PCS1, 2, and 7 showed the same host range pattern (A), and vB_Pfl?-PCS3 and 6 showed the same host range pattern (D). Since the phages were isolated from the different chicken samples purchased at the different supermarket at different day, it seems that they were different phages. Genomic DNA sequencing is required to confirm that these phages showing the same host range pattern are different phages.

Among *Pseudomonas* phages, some phages, such as *P. tolaasii* phage Bf7 (Sajben-Nagy et al. 2012), have been reported to have wide host specificity, infecting multiple *Pseudomonas* spp., including *P. agarici*, *P. costantinii*, *P. fluorescens*, *P. fluorescens*, *P. putida*, and *P. tolaasii*. In this study, phage vB_PflP-PCS4 and vB_PflP-PCW2 infected 77.8% and 44.4% of the *Pseudomonas* strains selected from food-spoilage pseudomonads, respectively (Fig. 1). The combined use of these two phages did not extend the host range but enhanced lytic activity and effectively retarded or inhibited regrowth of the phage-resistant population in both strains of the *P. fluorescens* group and *P. putida* at 30 °C, 8 °C, and 4 °C, although the single phage did not retard the regrowth of bacteria. Since these phages seem to have great potential for the biocontrol of pseudomonads during cold storage of food, the effect of the phage cocktail on the viability and growth of pseudomonads in various foods during cold storage will be reported in the near future. In actual food application tests, it is necessary to conduct tests with wide ranges of MOIs to determine the optimal phage application conditions.

Phage vB_PflP-PCS4 genome showed 97.8% similarity to *P. fluorescens* phage UNO-SLW1 genome. However, the tail fiber gene showed 53.55% identity to that of

Pseudomonas phage UNO-SLW1, being the lowest similarity to the known gene among the whole genes in phage vB_PflP-PCS4 (Table S2). The phage tail fiber plays a very important role in binding to the host bacterial phage receptor. This result suggests that vB_PflP-PCS4 seems to be a novel phage with a different host range from the previously reported *Pseudomonas* phages. Phage vB_PflP-PCW2 genome had 98.04% similarity with the *P. syringae* pv. Actinidiae phage CHF7 complete genome. In contrast to phage vB_PflP-PCS4, the tail fiber gene showed high similarity to that of *Pseudomonas* phage CHF21, but low similarity in tail fiber assembly chaperone gene (67.23% identity to tail fiber assembly chaperone of *Pseudomonas* phage vB_PsyP_3MF5) and DNA-directed RNA polymerase gene (61.36% identity to hypothetical protein of *Pseudomonas* phage ALEA) and HNH endonuclease gene (58.39% identity to hypothetical protein of *Pseudomonas* phage CHF1) (Table S3), suggesting phage vB_PflP-PCW2 seems to be a novel phage with similar host range of known phages.

Genome analysis of vB_PflP-PCS4 and vB_PflP-PCW2 revealed no tRNA, virulence, or toxicity genes, suggesting that they can be used in food. Moreover, phages vB_PflP-PCS4 and vB_PflP-PCW2 harbored a peptidoglycan lytic exotransglycosylase gene (EC:4.2.2. n1) as the tail spike protein and N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28) as the endolysin. Phage vB_PflP-PCS4 had a gene encoding a novel N-acetylmuramoyl-L-alanine amidase endolysin (gpp19), which showed 87.58% identity to the lysin of *P. fluorescens* phage UNO-SLW1 (accession code YP_009793708). In addition, the RZ lysis protein or spanin (gpp43) of phage vB_PflP-PCS4 showed 97.95% identity to the putative Rz lysis protein of *Pseudomonas* phage UNO-SLW1 (accession code YP_009793733.1) and low identity (61.54%) to the lysis protein of *Pseudomonas* phage PFP1 (YP_009804024.1). RZ lysis protein or spanin is a bacteriolytic enzyme required for disrupting the outer membrane and is present in most gram-negative bacteriophages (Summer et al. 2007). Spanins are cationic antimicrobial peptides (Holt et al. 2021). Based on these facts, further characterization of vB_PflP-PCS4 lysis proteins expressed as recombinant proteins is needed to apply the enzymes to control gram-negative bacteria in food matrices.

Lytic activity tests of phages at low temperatures showed that phages PCL1 and PSP1 showed strong lytic activity at 4 °C (Fig. 3). Some *Pseudomonas* phages have been reported to be active at low temperatures, such as *P. fluorescens* phage VW-6S and VW-6B (Xiang et al. 2018). Moreover, considering the possibility that the surface structure and metabolism of bacteria are different between low and optimal growth temperatures (Moreno and Rojo, 2014), the lytic activity of the phage cocktail was investigated at 4 °C and 8 °C (Fig. 7). Even at low temperatures, the phage cocktail showed strong lytic activity and retarded or inhibited the

regrowth of *P. fluorescens* group No. 271 and *P. putida*, suggesting its effectiveness in extending the shelf life of food under cold storage. Further experiments will be performed to investigate the effects of this phage cocktail on the viability and regrowth of various food-spoilage pseudomonads in food matrices at low temperatures.

The combination of vB_PflP-PCS4 and vB_PflP-PCW2 phages caused rapid lysis of the host bacterium after 1 h of incubation at 30 °C, 8 °C, and 4 °C (Figs. 5 and 7). In general, the combined effect of multiple phages is explained by differences in receptor-binding proteins (RBPs), and many phage cocktails have been designed focusing on differences in host range or RBPs. However, some reports have shown that the efficacy of phage cocktails differs depending on the MOI, temperature, incubation time, and phage-derived lysis proteins (Niu et al. 2021). Lysis proteins, such as tail spike proteins and endolysins, are characterized by their ability to lyse bacterial cells more rapidly and cause less bacterial resistance than phages (Schmelcher et al. 2012). Therefore, combining different endolysins and tail spike proteins derived from phages vB_PflP-PCS4 and vB_PflP-PCW2 caused rapid lysis and suppressed bacterial regrowth. In future studies, these proteins derived from phages will be produced as recombinant proteins, and the mechanism underlying the rapid lysis of bacterial cells by the phage cocktail will be investigated.

Conclusions

Among the 12 *Pseudomonas* phages isolated from chicken meat and soil samples, a phage cocktail consisting of phages vB_PflP-PCS4 and vB_PflP-PCW2 showed strong lytic activity against lettuce-derived *Pseudomonas* spp. and *P. putida* NBRC14164 at low temperatures. The absence of tRNA, virulence, or toxicity genes in the genomic DNA of these phages suggests that they are safe for use in food. The phage cocktail seems potentially effective as an antimicrobial agent against the psychrotrophic spoilage *Pseudomonas* spp. in food. Genomic DNA sequence analysis indicated that vB_PflP-PCS4 harbors a novel endolysin gene. Further research is necessary to reveal the mechanisms underlying the combined effects of phages vB_PflP-PCS4 and vB_PflP-PCW2, characterize the novel endolysin, and evaluate the lytic activity and growth inhibition of phage-resistant bacteria using the phage cocktail in food.

Data availability The authors confirm that the data supporting the findings of this study are available in the article and its supplementary information.

Declarations

Competing interests The authors declare no competing interests.

References

Bunker ST, Bates TC, Oliver JD (2004) Effects of temperature on detecting plasmid or chromosomally encoded *gfp*-and *lux*-labeled *Pseudomonas fluorescens* in soil. Environ Biosaf Res 3:83–90. https://doi.org/10.1051/ebr:2004008

Ceyssens PJ, Lavigne R (2010) Bacteriophages of *Pseudomonas*. Future Microbiol 5:1041–1055. https://doi.org/10.2217/fmb.10.66

Chan PP, Lin BY, Mak AJ, Lowe TM (2021) TRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. Nucleic Acids Res 49:9077–9096. https://doi.org/10.1093/nar/gkab688

Clausen PTLC, Aarestrup FM, Lund O (2018) Rapid and precise alignment of raw reads against redundant databases with KMA. BMC Bioinforma 19:1–9. https://doi.org/10.1186/s12859-018-2336-6

Cornelissen A, Ceyssens PJ, Krylov VN, Noben JP, Volckaert G, Lavigne R (2012) Identification of EPS-degrading activity within the tail spikes of the novel *Pseudomonas putida* phage AF. Virology 434:251–256. https://doi.org/10.1016/j.virol.2012.09.030

Endersen L, Coffey A (2020) The use of bacteriophages for food safety. Curr Opin Food Sci 36:1–8. https://doi.org/10.1016/j.cofs.2020.10.006

Fanelli F, Caputo L, Quintieri L (2021) Phenotypic and genomic characterization of *Pseudomonas putida* ITEM 17297 spoiler of fresh vegetables: focus on biofilm and antibiotic resistance interaction. Curr Res Food Sci 4:74–82. https://doi.org/10.1016/j.crfs.2021.02.001

Flores O, Retamales J, Núñez M, León M, Salinas P, Besoain X, Yañez C, Bastías R (2020) Characterization of bacteriophages against *Pseudomonas syringae* pv. Actinidiae with potential use as natural antimicrobials in kiwifruit plants. Microorganisms 8(7):974. https://doi.org/10.3390/microorganisms8070974

Hirano SS, Upper CD (2000) Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae* —a pathogen, ice nucleus, and epiphyte. Microbiol Mol Biol Rev 64:624–653. https://doi.org/10.1128/mmbr.64.3.624-653.2000

Holt A, Cahill J, Ramsey J, Martin C, O'Leary C, Moreland R, Maddox LT, Galbadage T, Sharan R, Sule P, Cirillo JD, Young R (2021) Phage-encoded cationic antimicrobial peptide required for lysis. J Bacteriol 204(1):e00214-221. https://doi.org/10.1128/jb.00214-21

Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM (2014) Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. J Clin Microbiol 52:1501–1510. https://doi.org/10.1128/JCM.03617-13

Knecht LE, Veljkovic M, Fieseler L (2020) Diversity and function of phage encoded depolymerases. Front Microbiol 10:1–16. https://doi.org/10.3389/fmicb.2019.02949

Kosugi S, Hirakawa H, Tabata S (2015) GMcloser: closing gaps in assemblies accurately with a likelihood-based selection of contig or long-read alignments. Bioinformatics 31:3733–3741. https://doi.org/10.1093/bioinformatics/btv465

Kropinski AM, Prangishvili D, Lavigne R (2009) Position paper: the creation of a rational scheme for the nomenclature of viruses of Bacteria and Archaea. Environ Microbiol 11:2775–2777. https://doi.org/10.1111/j.1462-2920.2009.01970.x

Lammens EM, Nikel PI, Lavigne R (2020) Exploring the synthetic biology potential of bacteriophages for engineering nonmodel bacteria. Nat Commun 11:5294. https://doi.org/10.1038/s41467-020-19124-x

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematic. John Wiley and Sons, New York, pp 115–175

Lister PD, Wolter DJ, Hanson ND (2009) Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 22:582–610. https://doi.org/10.1128/CMR.00040-09

Lowe TM, Chan PP (2016) tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44:W54–W57. https://doi.org/10.1093/nar/gkw413

Lu G, Luhr J, Stoecklein A, Warner P, Tapprich W (2017) Complete genome sequences of *Pseudomonas fluorescens* bacteriophages isolated from freshwater samples in Omaha, Nebraska. Genome Announc 5(12):e01501-e1516. https://doi.org/10.1128/genomeA.01501-16

Malberg Tetzschner AM, Johnson JR, Johnston BD, Lund O, Scheutz F (2020) In Silico Genotyping ofE scherichia coli Isolates for Extraintestinal Virulence Genes by Use of Whole-Genome Sequencing Data. J Clin Microbiol 58(10):e01269-20. https://doi.org/10.1128/JCM.01269-20

Masuda Y, Kawabata S, Uedoi T, Honjoh KI, Miyamoto (2021) Construction of leaderless-bacteriocin-producing bacteriophage targeting *E. coli* and neighboring gram-positive pathogens. Microbiol Spectr 9(1):e0014121. https://doi.org/10.1111/1758-2229.12150

Naknaen A, Samernate T, Wannasrichan W (2023) Combination of genetically diverse *Pseudomonas* phages enhances the cocktail efficiency against bacteria. Sci Rep 13:8921. https://doi.org/10.1038/s41598-023-36034-2

Nishimori E, Kita-Tsukamoto K, Wakabayashi H (2000) *Pseudomonas plecoglossicida* sp. nov., the causative agent of bacterial hemorrhagic ascites of ayu, *Plecoglossus altivelis*. Int J Syst Evol Microbiol 50:83–89. https://doi.org/10.1099/00207713-50-1-83

Niu YD, Liu H, Du H, Meng R, Sayed Mahmoud E, Wang G, McAllister TA, Stanford K (2021) Efficacy of individual bacteriophages does not predict efficacy of bacteriophage cocktails for control of *Escherichia coli* O157. Front Microbiol 12:616712. https://doi.org/10.3389/fmicb.2021.616712

Papadopoulou OS, Iliopoulos V, Mallouchos A, Panagou EZ, Chorianopoulos N, Tassou CC, Nychas G-J (2020) Spoilage potential of *Pseudomonas* (*P. fragi, P. putida*) and LAB (*Leuconostoc mesenteroides, Lactobacillus sakei*) strains and their volatilome profile during storage of sterile pork meat using GC/MS and data analytics. Foods 9:633

Peng X, Nguyen A, Ghosh D (2018) Quantification of M13 and T7 bacteriophages by TaqMan and SYBR Green qPCR. J Virol Methods 252:100–107. https://doi.org/10.1016/j.jviromet.2017.11.012

Ramsay L, Macaulay M, Degli Ivanissevich S, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R

(2000) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Genetics 156:1997–2005. https://doi.org/10.1093/genetics/156.4.1997

Raposo A, Pérez E, dFaria CT, Ferrús MA, Carrascosa C (2017) Food Spoilage byP seudomonas spp.—An overview. In: Singh OV (ed) Foodborne Pathogens and Antibiotic Resistance. Wiley, Hoboken, pp 41–58. https://doi.org/10.1002/9781119139188.ch3

Sajben-Nagy E, Maróti G, Kredics L, Horváth B, Párducz Á, Vágvölgyi C, Manczinger L (2012) Isolation of new *Pseudomonas tolaasii* bacteriophages and genomic investigation of the lytic phage BF7. FEMS Microbiol Lett 332:162–169. https://doi.org/10.1111/j.1574-6968.2012.02592.x

Scales BS, Dickson RP, Lipuma JJ, Huffnagle GB (2014) Microbiology, genomics, and clinical significance of the *Pseudomonas fluorescens* species complex, an unappreciated colonizer of humans. Clin Microbiol Rev 27:927–948. https://doi.org/10.1128/CMR.00044-14

Schmelcher M, Donovan DM, Loessner MJ (2012) Bacteriophage endolysins as novel antimicrobials. Future Microbiol 7:1147–1171. https://doi.org/10.2217/fmb.12.97

Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153

Simões M, Simões LC, Vieira MJ (2010) A review of current and emergent biofilm control strategies. LWT - Food Sci Technol 43:573–583. https://doi.org/10.1016/J.LWT.2009.12.008

Son HM, Duc HM, Masuda Y, Honjoh K, Miyamoto T (2018) Application of bacteriophages in simultaneously controlling *Escherichia coli* O157:H7 and extended-spectrum beta-lactamase producing *Escherichia coli*. Appl Microbiol Biotechnol 102:10259–10271. https://doi.org/10.1007/s00253-018-9399-1

Sulakvelidze A, Alavidze, Morris J (2001) Bacteriophage therapy. Antimicrob Agents Chemother 45:649–659. https://doi.org/10.1128/AAC.45.3.649-659.2001

Summer EJ, Berry J, Tran TAT, Niu L, Struck DK, Young R (2007) Rz/Rz1 lysis gene equivalents in phages of gram-negative hosts. J Mol Biol 373:1098–1112. https://doi.org/10.1016/j.jmb.2007.08.045

Sutherland IW, Hughes KA, Skillman LC, Tait K (2004) The interaction of phage and biofilms. FEMS Microbiol Lett 232(1):1–6. https://doi.org/10.1016/S0378-1097(04)00041-2

Tanaka C, Yamada K, Takeuchi H, Inokuchi Y, Kashiwagi A, Toba T (2018) A lytic bacteriophage for controlling *Pseudomonas lactis* in raw cow's milk. Appl Environ Microbiol 84(18):e00111-e118. https://doi.org/10.1128/AEM.00111-18

Thiel K (2004) Old dogma, new tricks - 21st century phage therapy. Nat Biotechnol 22:31–36. https://doi.org/10.1038/nbt0104-31

Xiang Y, Wang S, Li J, Wei Y, Zhang Q, Lin L, Ji X (2018) Isolation and characterization of two lytic cold-active bacteriophages infecting *Pseudomonas fluorescens* from the Napahai plateau wetland. Can J Microbiol 64:183–190. https://doi.org/10.1139/cjm-2017-0572

Zerbino DR, Birney E (2008) Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:821–829. https://doi.org/10.1101/gr.074492.107