수의학석사 학위논문

Phage-Based Biocontrol against Blight of *Rosaceae* Plant Caused by *Erwinia amylovora* and *Erwinia pyrifoliae*

에르위니아 아밀로보라와 에르위니아
피리폴리애에 의해 발생하는 장미과 식물의
마름병에 대한 파지 기반 생물학적 방제법 개발

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수의학과 수의병인생물학 및 예방수의학 전공

조 수 진

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By

Su Jin Jo

August, 2023

Veterinary Pathobiology and Preventive Medicine

Department of Veterinary Medicine

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By

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Major in Veterinary Pathobiology and Preventive Medicine
Department of Veterinary Medicine
Graduate School of Seoul National University

Abstract

Phage-Based Biocontrol against Blight of *Rosaceae* Plant Caused by *Erwinia amylovora* and *Erwinia pyrifoliae*

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The recent outbreak of blight in pome fruit plants has been a major concern as there are two indistinguishable *Erwinia* species, *Erwinia amylovora* and *E. pyrifoliae*, which cause blight in South Korea. Although there is a strict management protocol consisting of antibiotic-based prevention, the area and number of cases of outbreaks have increased. In this study, we isolated four bacteriophages (phages), pEp_SNUABM_03, 04, 11, and 12, that infect

both *E. amylovora* and *E. pyrifoliae* and evaluated their potential as antimicrobial agents for administration against *Erwinia*-originated blight in South Korea. Morphological analysis revealed that all phages had podovirus-like capsids. The phage cocktail showed a broad spectrum of infectivity, infecting 98.91% of *E. amylovora* and 100% of *E. pyrifoliae* strains. The antibacterial effect was observed after long-term cocktail treatment against *E. amylovora*, whereas it was observed for both short- and long-term treatments against *E. pyrifoliae*. Genomic analysis verified that the phages did not encode harmful genes such as antibiotic resistance or virulence genes. All phages were stable under general orchard conditions. Collectively, we provided basic data on the potential of phages as biocontrol agents that target both *E. amylovora* and *E. pyrifoliae*.

Keywords: Bacteriophage; *Erwinia* blight; pome fruit; phage cocktail; agriculture

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Contents

Abbreviations

ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
CFU	Colony Forming Unit
EDTA	Ethylenediaminetetraacetic acid
MOI	Multiplicity of Infection
NA	Nutrient Agar
NB	Nutrient Broth
ORF	Open Reading Frame
PFU	Plaque Forming Unit
RAST	Rapid Annotation using Subsystem Technology
SDS	Sodium dodecyl sulfate
SM	Sodium-Magnesium
TEM	Transmission Electron Microscope
VICTOR	Virus Classification and Tree Building Online Resource

Introduction

A major pathogenic bacterium of the pome fruit plant, *Erwinia amylovora*, has recently been introduced into South Korea [1-3]. *E. amylovora* has been reported to result in symptoms indistinguishable from those of *E. pyrifoliae*, an endemic pathogen in South Korea [4-6]. Both pathogens cause blight disease with blackening of leaves, stems, and immature fruits, starting with flower infection [7-10]. As *E. amylovora* is regulated by law, the disease management protocol should be performed in a different way compared to *E. pyrifoliae* outbreaks [11]. Therefore, strict regulations are applied to *E. amylovora* outbreaks, with orchards being forcibly closed at 5% outbreak rates (or less) with the discretion of the government plant-disease control agent [12, 13].

Periodic surveillance and prevention-based disease control programs must be performed to prevent the spread of these two pathogens [14]. The general protocol for fire blight prevention consists of three antibiotic administrations (once before flowering and twice during the flowering period). To prevent black shoot blight, antibiotics are administered twice after full bloom [12, 15]. Despite the intensive disease control program and antibiotics for *Erwinia*-associated blight, the outbreak of fire blight has been on the rise, with an increased possibility of evolution

of antibiotic resistance among pathogenic strains [16, 17]. Therefore, it is necessary to develop more effective agents other than antibiotics for the treatment of pathogenic *Erwinia* species [18, 19].

Bacteriophages have been used as effective antimicrobial agents for the treatment of fire blight worldwide [20, 21]. Phages are “smart biocontrol agents” as they replicate at the targeted infection site, enabling prolonged antimicrobial effects on-site [22, 23]. The infection specificity of phages allows specific pathogens to be targeted while maintaining beneficial microbes in the environment [24, 25]. To maximize the antimicrobial effects of phages, a combination of phages with different host spectra is used to exert antimicrobial effects over a wider range of pathogens; this pret-a-porter approach is one of the main paradigms for therapeutic phage preparation [26, 27]. Furthermore, cocktail phage therapy, which is a combinatorial strategy, has been reported to have a synergistic effect [28-31].

This study investigated the biological control potential of the newly isolated *Erwinia* phages. The biological and genomic characteristics, including morphology, stability, and antimicrobial potential of four phages that showed infectivity toward both *E. amylovora* and *E. pyrifoliae* were examined in this study.

Materials & Methods

Phage isolation

Water and soil samples were collected near the location where the blight outbreak occurred in South Korea to isolate phages that infect *E. pyrifoliae*. Phages were isolated as previously described [32]. Distilled water (10 mL) was added to the soil samples (1g). The samples were centrifuged at 10,000 × g for 10 min to remove contaminants. A host strain suspension (1%, v/v) containing *E. amylovora* (TS3128) or *E. pyrifoliae* (KACC13945) was cultured overnight for approximately 18 h at 27 °C. The suspension was used to inoculate the samples and nutrient broth (NB; Difco) for phage enrichment and cultured for 24 h at 27 °C. After enrichment, serial dilutions of the culture broth were transferred onto bacterial lawns of the *E. amylovora* (TS3128) or *E. pyrifoliae* (KACC13945). Phage isolation was confirmed using a double layer agar assay. The double layer agar assay was used to verify bacteriolysis induced by the inhibition spots of phages. The samples showing plaque formation were centrifuged at 10,000 × g and passed through 0.45-μm syringe filters. Pure phages were obtained by picking a single plaque and subjecting it to double layer assay five times.

Phage propagation and purification

Phage propagation was conducted as previously described [33]. The overnight culture (1%) was inoculated with different multiplicity of infection (MOIs; 10, 5, 1 and 0.1) of phages to determine the optimum ratio for phage propagation and cultured for 24 h at 27 °C. Phage lysate was centrifuged at 12,000 × g for 10 min and the supernatant was precipitated with 10% (w/v) polyethylene glycol/ 0.5 M NaCl. (Final concentration). A cesium chloride (CsCl) gradient was used to purify the phage suspension. The gradient layers were ultracentrifuged at 182,000 × g for 3 h. Phage precipitation bands were collected and dialyzed using a dialysis bag (Slide-A-Lyzer™ Dialysis Cassettes, 10,000 MWCO).

Transmission electron microscopy (TEM)

Purified phage suspensions (10 μL) were mixed with the same volume of uranyl acetate (2%). The suspensions were incubated on a copper grid for 1 min. Excess sample was removed and washed with distilled water. Images of the phages were obtained using a Talos L120C (FEI, USA) at 120 kV. The dimensions of four independent phages were determined (n = 5).

Host range

All the bacterial strains used in host range assay were recent isolates from the blight tissues in South Korea. A total of 116 bacterial strains,

including 92 *E. amylovora* and 24 *E. pyrifoliae* strains were spot assayed on nutrient agar (NA; Difco) plates with serial dilutions (10^{-1} to 10^{-8}) of purified phage suspension; the plates were incubated for 24 h at 27 °C [40]. Plaque formation on the spot areas resulted in the bacterial strain being considered susceptible and is represented as "+" in Table 1. The experiments were performed in triplicates.

Stability test

Thermal stability of the phages was evaluated as described by Kim et al. [35]. Phage suspensions (1 mL each, 2×10^8 PFU/mL) were incubated for 60 min at 4 (control), 20, 30, 40, and 50 °C. Approximately 100 μL aliquots of each suspension was used to determine the concentration of phages using a double layer agar assay. pH stability of the phages was evaluated by adjusting the pH of phage suspensions (2×10^8 PFU/mL) to 4.0, 5.0, 6.0, 7.0 (control), 8.0, and 9.0 with 0.1 M HCl and 0.1 M NaOH; each of the phage suspensions were then incubated for 60 min at 27 °C. They were then evaluated using a double layer agar assay. All tests were performed in triplicates.

One-step growth curve

The phage suspension (100 μL) was inoculated into 10 mL of

exponentially growing host strain culture (2×10^8 colony-forming units [CFU]/mL) at an MOI of 0.001 [36]. The phages were allowed to infect the bacterial cells for 10 min and the suspension was centrifuged at 12,000 × g to remove unattached phages. The phage-infected bacterial pellets were then resuspended in preheated NB (10 mL) and incubated at 27 °C with shaking (150 rpm). Aliquots (100 μL) were collected at 5 min intervals for 50 min; the titers were then evaluated using double layer agar assay. The experiments were performed in triplicates.

Genome analysis

Genomic DNA was extracted from phages as described previously [28, 32]. Purified phage suspension ($\geq 10^{10}$ PFU/mL) was digested with 10 IU of DNase I and RNase A to remove nucleotides originating from the hosts. The nucleases were heat-inactivated at 95 °C by the addition of EDTA. Proteinase K and SDS (10%) were added to the samples to degrade structural proteins. DNA was purified with phenol-chloroform-isopropanol and precipitated with absolute ethanol, followed by two washes with 70% ethanol. The phage genomic DNA was sequenced using an Illumina HiSeq platform at Macrogen (Seoul, South Korea). The short reads were assembled into contigs using de bruin graphs in CLC genomic workbench (v. 6.5.1). Open reading frames (ORFs) were identified using GenMarkS and Rapid

Annotation using subsystem Technology (RAST) [37, 38]. The presence of tRNA, and virulence and antibiotic genes was determined using tRNAscan-SE, VirulenceFinder, and ResFinder, respectively [39-41]. Comparative genome analysis was performed based on sequence similarity using tBLASTx [42]. Whole-genome phylogenetic analysis was performed using the Virus Classification and Tree Building Online Resource (VICTOR) with the recommended setting for complete nucleotide sequences [43].

Antibacterial activity

The antibacterial effect of pEp_SNUABM_03, 04, 11, and 12 was evaluated over short (2 h) and long (8 h) periods of time. The assay was performed using two indicator strains, *E. amylovora* (TS3128) and *E. pyrifoliae* (KACC13945). The phage cocktail was prepared by combining the four phages at equal ratio (1:1:1:1) to obtain 2×10^8 PFU/mL. Exponentially growing indicator strains were inoculated into fresh NB to obtain 2×10^5 CFU/mL for 8 h and at 27 °C, and the phage suspension was inoculated into the broth at three concentrations (MOI 5, 1, and 0.1). The mixtures were cultured with shaking at 150 rpm, and CFU were determined. The CFU values were determined by preparing serial dilutions in phosphate buffered saline and plating for quantification of viable bacteria. All tests were performed in triplicates.

Statistical analysis

Statistical differences were analyzed using Sigmaplot 12.5 (Systat Software Inc., IL, USA) using analysis of variance with the Holm-Sidak test. Statistical significance was set at $P < 0.05$.

Results

TEM – biological analysis

Morphological observations using TEM revealed four distinct phages that belong to *Podoviridae* (Figure 1). Structural observations of pEp_SNUABM_03, 04, 11, and 12 revealed short tails with head diameters of 56 ± 2, 55 ± 3, 56 ± 3, and 63 ± 2 nm (n = 5), respectively (Table 2).

Stability test

The test was conducted under normal-orchard environmental temperature and pH conditions (Figure. 2). Thermal stability tests showed that pEp_SNUABM_03 and 11 were stable at 4 (control), 20, 30, 40, and 50 °C for 1 h, and virions of pEp_SNUABM_04 were vulnerable to high temperature (50 °C; $P < 0.05$). The phage pEp_SNUABM_12 was sensitive to temperature changes ($P < 0.05$). The pH stability test revealed that pEp_SNUABM_04, 11, and 12 were all stable, whereas the stability of pEp_SNUABM_03 decreased at pH 9 ($P < 0.05$).

One-step growth curve

All four phages exhibited similar biological characteristics. Hence pEp_SNUABM_03 was used as a representative phage for one-step growth

analysis (Figure 3). After the 10 min latent period, the first burst size of the phage growth was 76.83 PFU per bacterial cell for pEp_SNUABM_03.

Genome analysis

The general characteristics of phages pEp_SNUABM_03, pEp_SNUABM_04, pEp_SNUABM_11, and pEp_SNUABM_12 are listed in Table 3. A total number of reads 3,864,800 (pEp_SNUABM_03), 3,730,842 (pEp_SNUABM_04), 3,426,138 (pEp_SNUABM_11), 3,818,762 (pEp_SNUABM_12) were obtained from the illumina sequencer, which was assembled into the single contig. The circular genomes of phages pEp_SNUABM_03, pEp_SNUABM_04, pEp_SNUABM_11, and pEp_SNUABM_12 contained 39,879, 39,649, 39,626, and 39,980 bp with GC contents of 52.13%, 52.19%, 52.10%, and 51.19%, respectively (Table 3). A total of 52, 52, 49, and 50 ORFs were identified in the genomes of pEp_SNUABM_03, pEp_SNUABM_04, pEp_SNUABM_11, and pEp_SNUABM_12, respectively. The function of the predicted ORFs was categorized into five groups: structural and packaging proteins, nucleotide metabolism-related proteins, lysis proteins, additional function proteins, and hypothetical proteins (Figure 4).

The phylogenetic positions of phages pEp_SNUABM_03, pEp_SNUABM_04, pEp_SNUABM_11, and pEp_SNUABM_12, which

have the morphology of podovirus, were analyzed using the complete genome sequences of closely related phages infecting *Enterobacterales* (*Erwinia*, *Dickeya*, and *Pectobacterium*). All phages were classified under the subfamily *Studiervirinae* in the family *Autographiviridae* (Figure 5). Phage pEp_SNUABM_12 clustered with *Ningirsuvirus* and the dickey phage Ninurta, whereas the other three phages were unclassified. Phages pEp_SNUABM_03, 04, and 11 were clustered with *Erwinia* phage vB_EamP-L1 belonging to *Elunavirus*. This cluster was most closely related to FE 44, another *Erwinia* phage belonging to *Berlinvirus*. Two clusters of the newly isolated phages branched from a common ancestor.

Comparative genome analysis supported the genomic distance between phages in the two clusters. The genomes of three unclassified phages, pEp_SNUABM_03, 04, and 11, showed highly conserved synteny revealing around 98% of nucleotide identity among them (thick blue), whereas the similarity level was low (nucleotide identity: around 70%; pale blue) with the closest neighbor, vB_EamP_L1 (Figure 6; Table 4). Phage pEp_SNUABM_12 showed high synteny with Ninurta (nucldotide identity: 94.66%), another member of *Ningirsuvirus* (Figure 6; Table 4) and genetic distance with pEp_SNUABM_03, 04, and 11. The three unclassified *Autographiviridae* phages shared more than 47 core genes, which accounted for more than 90% of their genes (Table 5). The shared genes among the

four phages isolated in this study decreased to only 37 genes, as revealed by the comparative blast analysis (Table 6, Table 7, Table 8, and Table 9).

Host range

Host range analysis was performed against 116 *Erwinia* strains including 92 *Erwinia amylovora* and 24 *Erwinia pyrifoliae* (Table 10). pEp_SNUABM_03 and 04 showed broad-host-spectrum infectivity to both *E. amylovora* (98.91%, 91/92; 97.83%, 90/92) and *E. pyrifoliae* (91.67%, 22/24; 95.83%, 23/24) strains, respectively. Although pEp_SNUABM_11 had a relatively narrow host range compared to pEp_SNUABM_03 and 04, it was highly infective (*E. amylovora*: 76.09%, 70/92; *E. pyrifoliae*: 79.17%, 19/24). Phage pEp_SNUABM_12 showed specific infectivity in *E. pyrifoliae* (95.83%, 23/24). pEp_SNUABM_12 was able to infect only two *E. amylovora* strains (2.17%, 2/92). The phage cocktail infected almost all *E. amylovora* (98.91%, 91/92) and *E. pyrifoliae* (100%, 24/24) strains.

Antibacterial activity of phages on E. amylovora

The antibacterial efficacy of the newly isolated phages was evaluated at three concentrations (MOI 0.1, 1, and 5) over short (2 h) and long (8 h) time periods. Phages pEp_SNUABM_03, 04, 11 and 12 co-cultured with *E. amylovora* TS3128 at an MOI of 0.1 resulted in a slight inhibition of

bacterial growth in the short term; pEp_SNUABM_04 showed significant inhibition after administration ($P < 0.05$). In the long term, the antibacterial effect was significant for all phages ($P < 0.001$), pEp_SNUABM_03 (-4.03 logCFU/mL), 04 (-3.70 logCFU/mL), 11 (-3.14 logCFU/mL), and 12 (-2.37 logCFU/mL). At an MOI of 1, all phages showed a significant inhibitory effect against TS3128 after short-term administration ($P < 0.05$). In the long term, all phages showed a significantly increased antibacterial effect, pEp_SNUABM_03 (-4.24 logCFU/mL), 04 (-3.78 logCFU/mL), 11 (-2.86 logCFU/mL), and 12 (-3.18 logCFU/mL) ($P < 0.001$). Phages pEp_SNUABM_03, 04, 11 and 12, were co-cultured with TS3128 at an MOI of 5 and exhibited a significant inhibition of bacterial growth in the short term for all phages ($P < 0.05$). In the long term, there were notable reductions in bacterial counts for all phages; pEp_SNUABM_03 (-4.24 logCFU/mL), 04 (-3.97 logCFU/mL), 11 (-2.77 logCFU/mL), and 12 (-3.29 logCFU/mL) ($P < 0.001$).

The phage cocktail consisted of equal ratio of the four phages, resulting in the same overall concentration as solely administered phages. Although one-fourth of each of the phages were combined, the antibacterial effect of the cocktail phage suspension administered over long term, -3.42 logCFU/mL (MOI 0.1), -3.93 log-CFU/mL(MOI 1), and -4.23 logCFU/mL (MOI 5), was higher than the average CFU reduction exhibited by

individual phages, which is indicative of a synergistic effect.

Antibacterial activity of phages on E. pyrifoliae

The antibacterial effects of the four phages were evaluated at three concentrations (MOI 0.1, 1, and 5) over short (2 h) and long (8 h) periods of time. All phages showed rapid antibacterial effects against *E. pyrifoliae*. When *E. pyrifoliae* KACC13945 and phages pEp_SNUABM_03, 04, 11, and 12 were co-cultured at an MOI of 0.1, bacterial growth was inhibited in the short term, with pEp_SNUABM_11 showing significant inhibition ($P < 0.05$). In the long term, the antibacterial effect significantly decreased for all phages ($P < 0.001$), pEp_SNUABM_03 (-5.17 logCFU/mL), 04 (-5.27 logCFU/mL), 11 (-4.43 logCFU/mL), and 12 (-5.10 logCFU/mL). At an MOI of 1, all phages rapidly inhibited bacterial growth after short-term administration and showed a significant inhibitory effect against KACC13945 ($P < 0.001$). In the long term, the antibacterial effect was sustained in all phages; pEp_SNUABM_03 (-5.33 logCFU/mL), 04 (-5.20 logCFU/mL), 11 (-3.19 logCFU/mL), and 12 (-5.07 logCFU/mL) ($P < 0.001$). Phages pEp_SNUABM_03, 04, 11, and 12 co-cultured with KACC13945 at an MOI of 5 showed considerable reductions in bacterial counts in the short term for all phages ($P < 0.001$). In the long term, the antibacterial effect was maintained, and the bacterial counts were

significantly reduced for all phages ($P < 0.001$); pEp_SNUABM_03 (-5.43 logCFU/mL), 04 (-5.17 logCFU/mL), 11 (-2.31 logCFU/mL), and 12 (-5.03 logCFU/mL).

The antibacterial efficacy of the phage cocktail suspension administered over a short term was -2.49 logCFU/mL (MOI 0.1), -3.03 logCFU/mL (MOI 1), and -3.77 logCFU/mL (MOI 5). Whereas the average CFU reduction of each phage, -2.50 logCFU/mL (MOI 0.1), -3.15 logCFU/mL (MOI 1), and -3.38 logCFU/mL (MOI 5), did not exhibit any synergy effect of the cocktail phage. However, there was a significant decrease in the bacterial count in the short-term phage cocktail treatment.

Discussion

Erwinia-associated blight disease in rosaceous fruit plants in South Korea is caused by *E. pyrifoliae* infection [5]. However, the recent outbreak of fire blight caused by *E. amylovora* has rendered the disease management protocol complicated, as a co-outbreak with *E. pyrifoliae* was identified [4, 44]. In contrast to *E. pyrifoliae*, fire blight caused by *E. amylovora* is registered as a legal communicable disease in plants in South Korea, and there is a distinct disease management protocol [10, 13]. To provide an effective control method against both pathogens, we isolated and characterized the potential of bacteriophages against *Erwinia*-originated blight disease in South Korea.

The rosaceous fruit plant industry has tried to use phages as biocontrol agents against *E. amylovora* outbreaks worldwide [45, 46]. A number of phages have been isolated, and their potential as antimicrobial agents has been confirmed [28, 47, 48]. A cocktail phage suspension that combines phages with different infection mechanisms is preferred over individual phage isolates to minimize resistance and maximize the antibacterial effect for effective disease control [28, 49]. As *Erwinia* bacteriophages have a broad host range, the major objective of their combined administration is to improve their antimicrobial potential [50, 30].

The four phages used in this study also had a broad host range, except for pEp_SNUABM_12, which specifically infects *E. pyrifoliae* (Table 10). Phages use distinct infection strategies based on their tail structure, and the infectivity of the four phages are distinct from each other [51, 52]. This suggests that they have different infection strategies that would prevent the prevalence of resistant bacterial strains [23, 53].

Several studies have shown that phage resistance in bacterial strains present in a form of trade-off [54, 55]; bacteria acquire phage resistance in return for fitness loss, including growth, virulence, and antibiotic susceptibility [56, 57]. Attenuation or loss of virulence has been observed in several strains of *Pectobacterium atrosepticum* and *Pseudomonas plecoglossicida* resistant against phages PPpW-3 and/or PPpW-4, respectively [58, 59]. Impaired growth characteristics have been reported in phage-resistant *E. amylovora* and *P. syringae*, which had significantly affected their virulence [60]. Phage-resistant *Escherichia coli*, and *E. amylovora* strains become more susceptible to antibiotics [28, 61]. Furthermore, *E. amylovora* bacteriophages showed transient resistance in infected bacterial strains, with phage infectivity being restored after the phage was eliminated.

Synergism is one of the major incentives for combining several phages in a cock-tail suspension [30]. A synergistic effect refers to the

antimicrobial potential of cocktail phages being greater than the sum of the individual phages; an additive effect occurs when a cocktail phage provides the sum of the effects of individual phages; an antagonistic effect refers to the antimicrobial potential of the cocktail phages being less than that of the sum of the individual phages [62]. The best selection for phage cocktail components results in synergy; as observed in our study (Figure 7), there should be no antagonistic effect between the cocktail phages. As phages can interrupt secondary infections by closely related phages, it is recommended that antagonistic phages be excluded at the first selection step.

The stability of phages under environmental stress should be verified before their application. The major stress factors expected are acidity, temperature, and UV radiation [63]. Although increased stability of the phages better facilitates their application as biocontrol agents, there are several ways to bypass environmental stresses (Figure 2). Control agents can be administered in the morning or encapsulated to minimize exposure to temperature and light, or acidity, respectively [64, 65].

Although the efficacy and stability of phages are guaranteed, safety is a major concern. Generally, phages with an obligatory lytic life cycle are preferred as biocontrol agents against *Erwinia*-originated blight diseases (Figure 4). On the other hand, lysogenic phages have greater potential of transducing harmful genes including those associated with antimicrobial

resistance, virulence, and toxins. However, if the transduction issue is eliminated, lysogenic phages may also be good candidates for controlling fire blight [66].

In the present study, the efficacy of the four phages and the phage cocktail against *Erwinia* strains indicates its possible use as a biocontrol agent under field conditions. The antibacterial effect can be further improved through modifications in the cocktail ratio as the phages exhibited synergy. To be applied in the actual environment, future studies should focus on the biocontrol efficacy of optimum phage cocktails in planta and carry out acute ecotoxic tests in fish to rule out possible environmental health hazards.

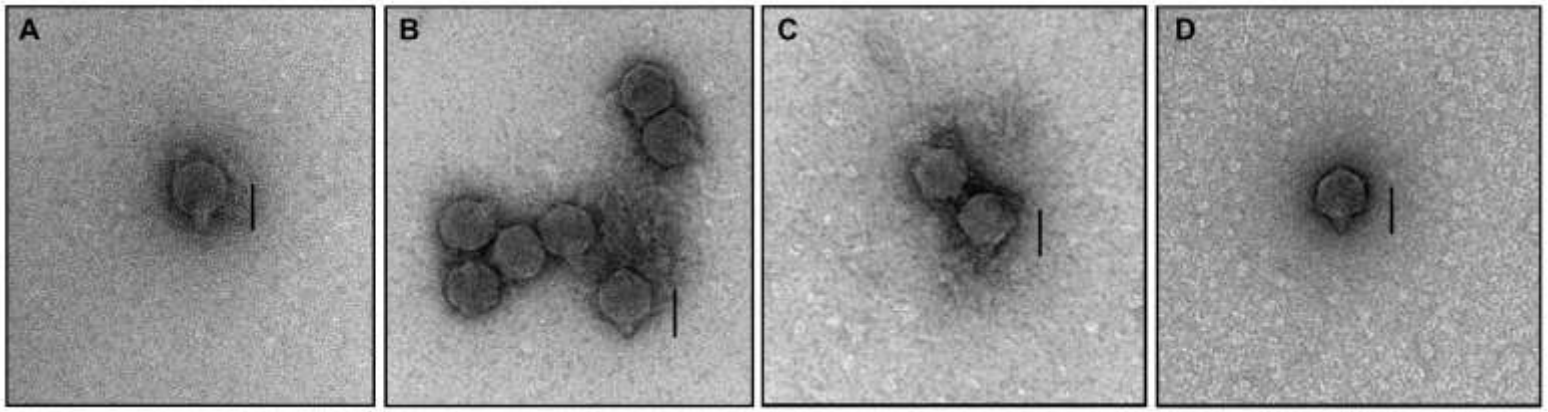

Figure 1. Morphological observation by transmission electron micrographs of *Erwinia pyrifoliae* phages (A) pEp_SNUABM_03, (B) pEp_SNUABM_04, (C) pEp_SNUABM_11, and (D) pEp_SNUABM_12. Scale bar = 50 nm.

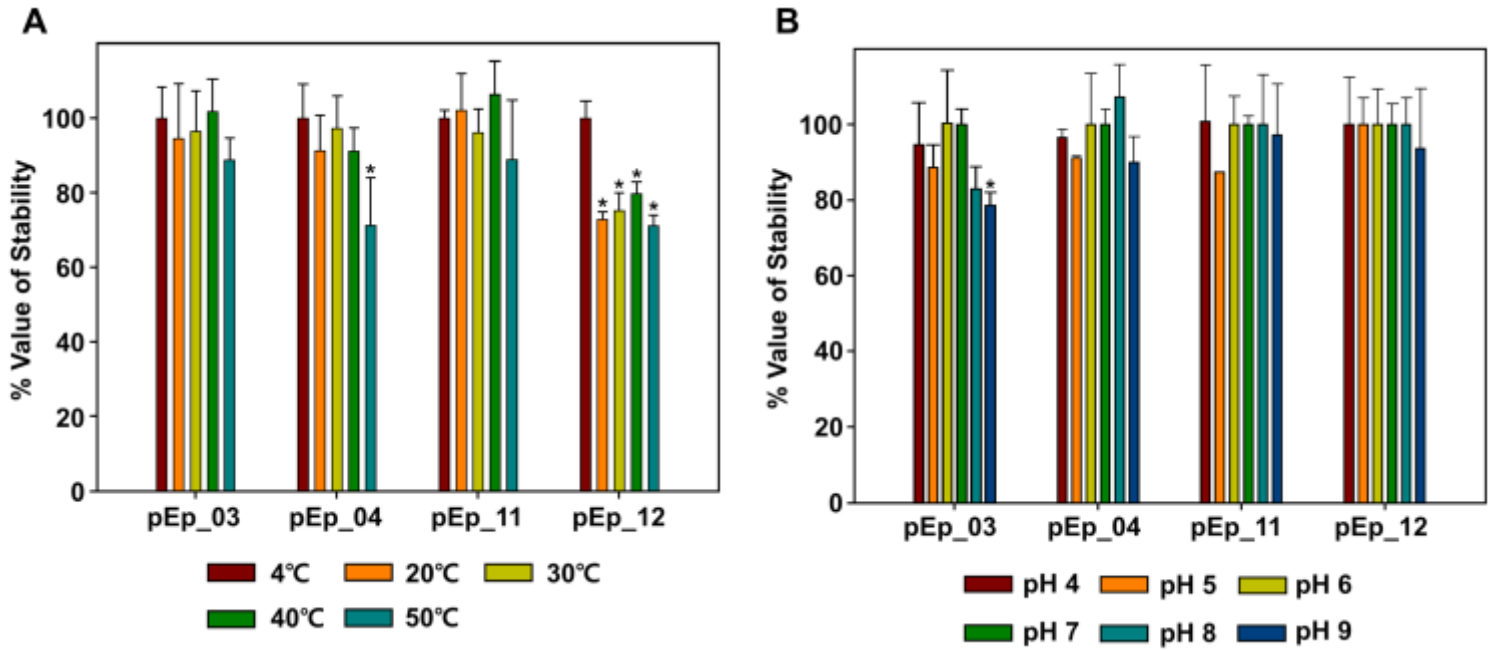

Figure 2. Stability of phages pEp_SNUABM_03, pEp_SNUABM_04, pEp_SNUABM_11, and pEp_SNUABM_12 at thermal (A) and pH (B) stresses. Phages were incubated for 1 h under each condition and the phage titer was determined on the host strain. One-way ANOVA with Holm-Sidak tests were performed to determine significant differences ($P < 0.05$). n = 3

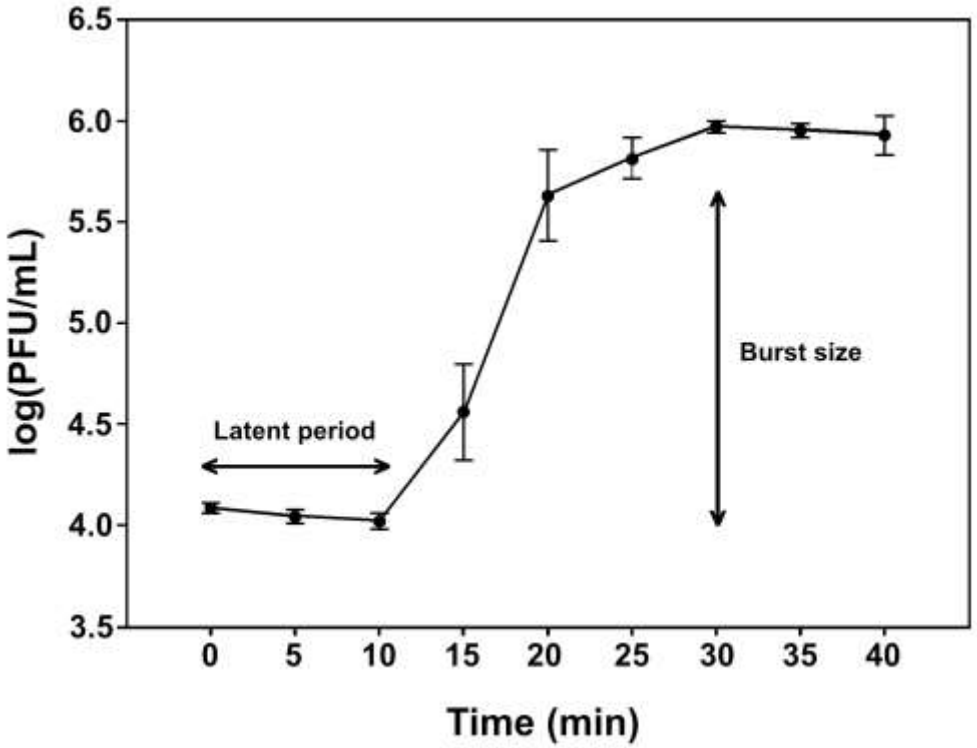

Figure 3. One-step growth curve of the pEp_SNUABM_03 in *E. pyrifoliae* strain KACC13945. The values are presented as mean ± standard deviation.

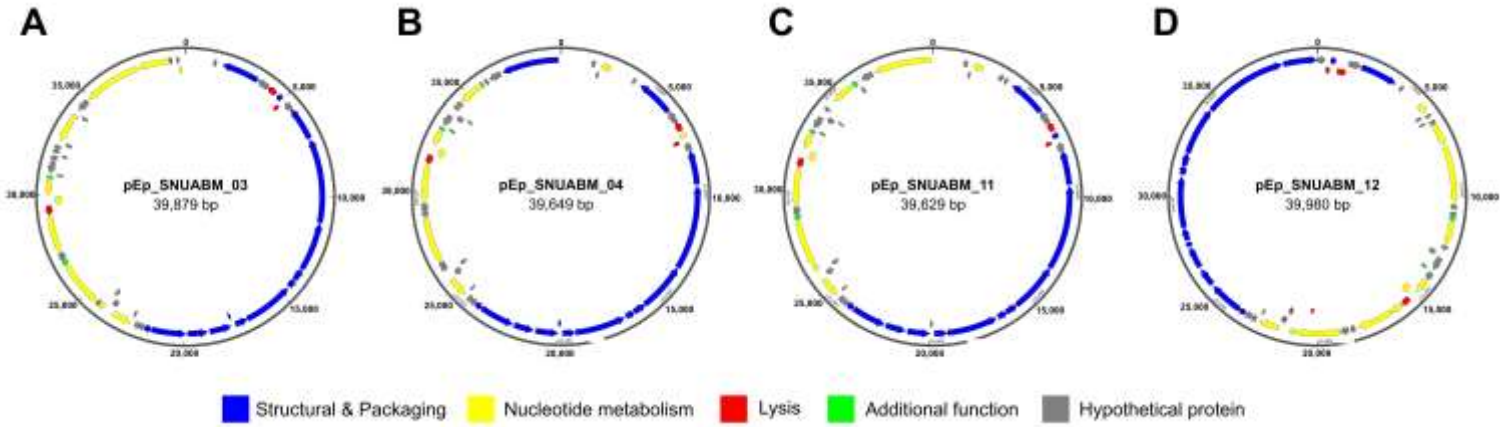

Figure 4. Genome map of *Erwinia* phages (A) pEp_SNUABM_03, (B) pEp_SNUABM_04, (C) pEp_SNUABM_ 11, and (D) pEp_SNUABM_12. The color-coded ORFs are classified based on their function (Scale = base pair).

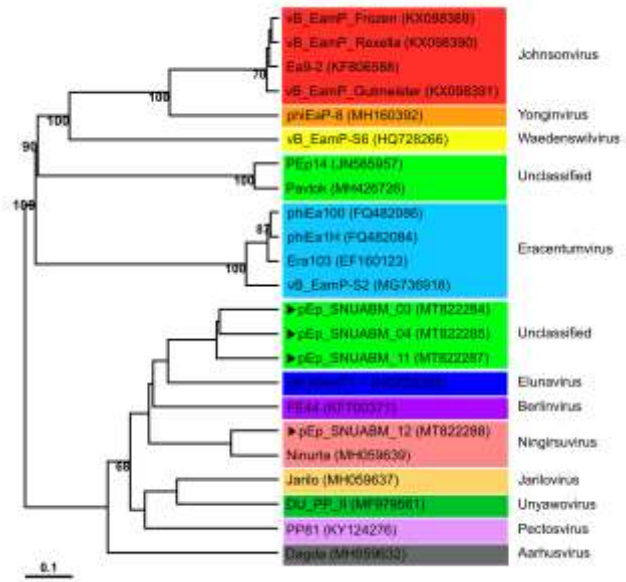

Figure 5. Whole-genome phylogenetic analysis of newly isolated *Erwinia* phages. The four phages isolated in this study are indicated with arrows (▶). The different genera (*Johnsonvirus*, red box; *Yonginvirus*, orange box, *Waedenswilvirus*, yellow box; unclassified, light green; *Eracentumvirus*, sky-blue box; *Elunavirus*, deep blue box; *Berlinvirus*, purple box; *Ningsuvirus*, pink box; *Jarilovirus*, light orange box; *Unyawovirus*, green box; *Pectosvirus*, purple box; and *Aarhusvirus*, gray box) are indicated using colors.

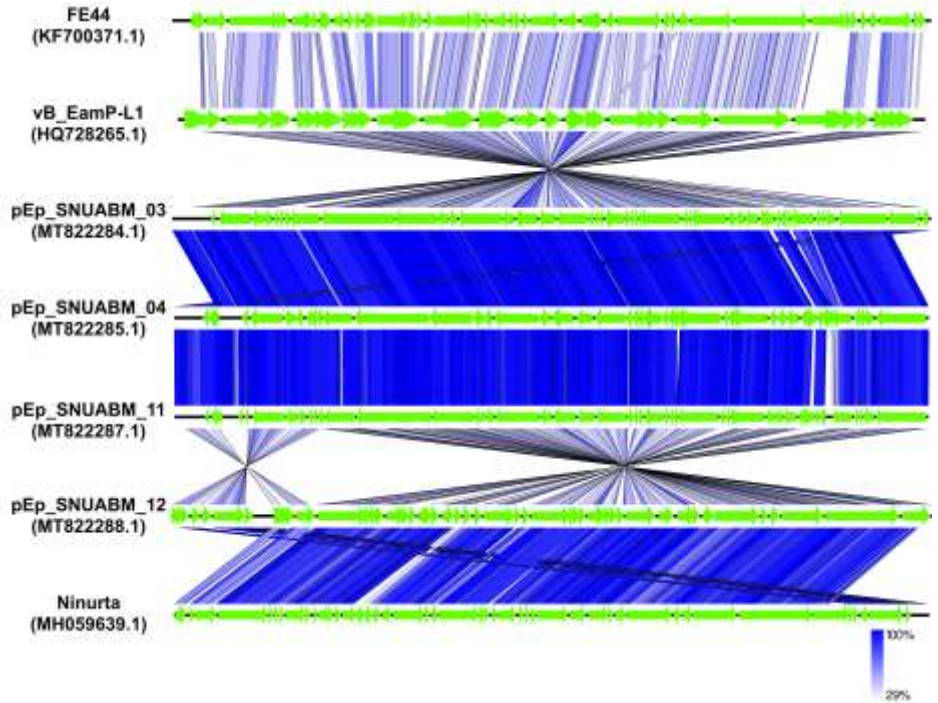

Figure 6. Comparative whole-genome analysis of *Erwinia* phages pEp_SNUABM_03, pEp_SNUABM_04, pEp_SNUABM_11, and pEp_SNUABM_12 among phages infecting *Enterobacterales* species. The tBLASTx comparison analysis was constructed with tBLASTx algorithm using Easyfig.

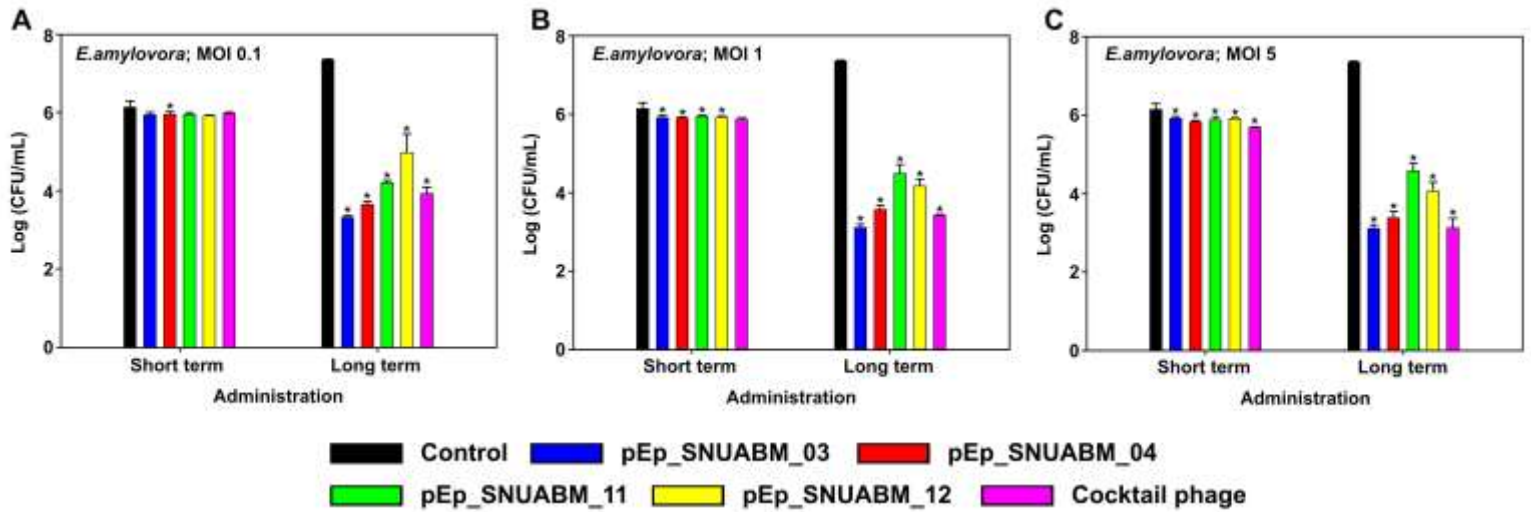

Figure 7. Evaluation of antibacterial activity of phages on *Erwinia amylovora*. The assay was performed at an MOI of 0.1 (A), 1 (B), and 5 (C). Statistical significance was calculated using a one-way analysis of variance (ANOVA) with Holm-Sidak tests ($P < 0.001$).

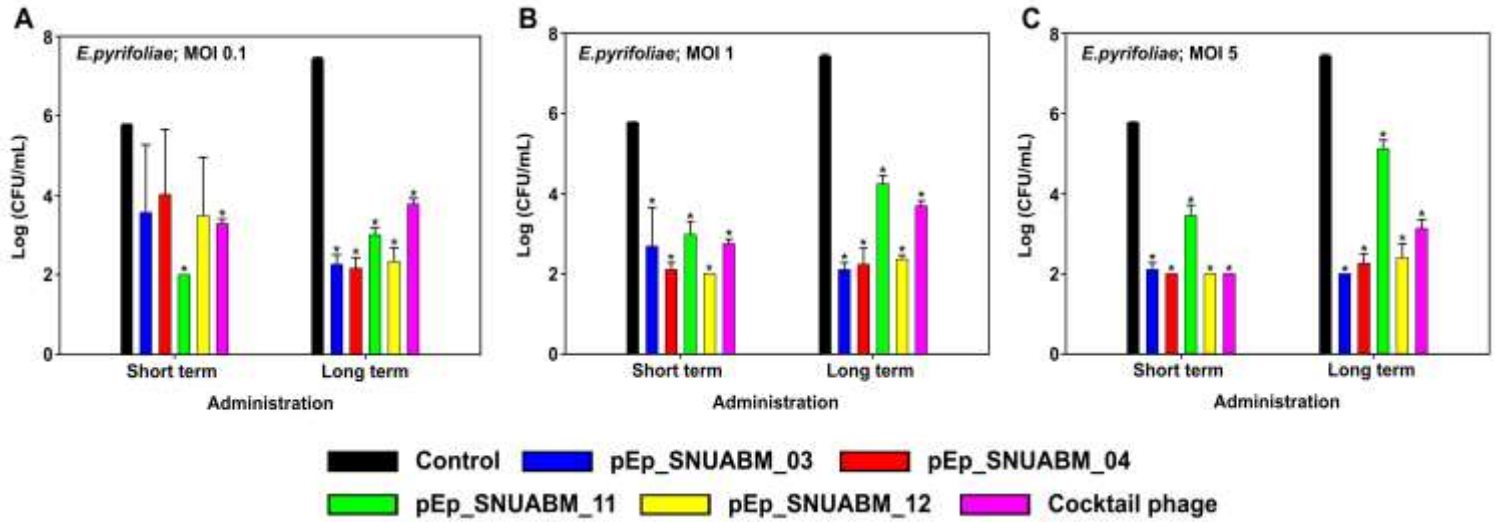

Figure 8. Evaluation of antibacterial activity of phages on *Erwinia pyrifoliae*. The assay was performed at an MOI of 0.1 (A), 1 (B), and 5 (C). Statistical significance was determined using a one-way analysis of variance (ANOVA) with Holm-Sidak tests ($P < 0.001$).

Table 1. Host range of phage pEp_SNUABM_03, pEp_SNUABM_04, pEp_SNUABM_11, pEp_SNUABM_12, and Cocktail phage (mixed pEp_SNUABM_03, 04, 11, 12) against *Erwinia amlyrovora* and *Erwinia pyrifoliae* strains used in this study.

species	strain	isolated		Phage infectivity				
		year	province	pEp_3	pEp_4	pEp_11	pEp_12	cocktail
Erwinia amlyrovora	YKB 14715	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14740	2019	Chungcheongbuk	+	+	-	-	+
	YKB 14742	2019	Chungcheongbuk	+	+	-	-	+
	YKB 14748	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14750	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14754	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14756	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14758	2019	Chungcheongbuk	+	+	+	-	+

	YKB 14768	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14770	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14776	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14778	2019	Chungcheongbuk	+	+	-	-	+
	YKB 14787	2019	Chungcheongnam	+	+	+	-	+
	YKB 14806	2019	Gyeonggi	+	+	-	-	+
	YKB 14808	2019	Gyeonggi	+	+	+	-	+
	YKB 14814	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14818	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14820	2019	Chungcheongbuk	+	+	+	-	+
	YKB 14822	2019	Chungcheongbuk	+	+	-	-	+
	RA0023	2020	Gyeonggi	+	+	+	-	+
	RA0024	2020	Gyeonggi	+	+	-	-	+
	RA0025	2020	Gyeonggi	+	+	+	-	+

	RA0026	2020	Gyeonggi	+	+	+	-	+
	RA0027	2020	Gyeonggi	+	+	+	-	+
	RA0028	2020	Gyeonggi	+	-	-	-	+
	RA0029	2020	Gyeonggi	+	+	+	-	+
	RA0030	2020	Gyeonggi	+	+	+	+	+
	RA0031	2020	Gyeonggi	+	+	+	-	+
	RA0032	2020	Gyeonggi	+	+	+	-	+
	RA0033	2020	Gyeonggi	-	-	-	-	-
	RA0034	2020	Gyeonggi	+	+	+	-	+
	RA0035	2020	Gyeonggi	+	+	+	-	+
	RA0036	2020	Gyeonggi	+	+	+	-	+
	RA0037	2020	Gyeonggi	+	+	+	-	+
	RA0038	2020	Jeollabuk	+	+	+	-	+
	RA0039	2020	Chungcheongnam	+	+	+	-	+

	RA0040	2019	Chungcheongnam	+	+	+	-	+
	RA0041	2019	Chungcheongnam	+	+	+	-	+
	RA0042	2020	Chungcheongnam	+	+	+	-	+
	RA0043	2020	Chungcheongnam	+	+	+	-	+
	RA0044	2020	Chungcheongnam	+	+	+	-	+
	RA0045	2020	Chungcheongbuk	+	+	+	-	+
	RA0046	2020	Chungcheongbuk	+	+	+	-	+
	RA0047	2020	Chungcheongbuk	+	+	+	-	+
	RA0048	2020	Chungcheongbuk	+	+	+	-	+
	RA0049	2020	Chungcheongbuk	+	+	+	-	+
	RA0050	2020	Chungcheongbuk	+	+	+	-	+
	RA0051	2020	Chungcheongbuk	+	+	+	-	+
	RA0052	2020	Chungcheongbuk	+	+	+	-	+
	RA0053	2020	Chungcheongbuk	+	+	+	-	+

	RA0054	2020	Chungcheongbuk	+	+	+	-	+
	RA0055	2020	Chungcheongbuk	+	+	+	-	+
	RA0056	2020	Chungcheongbuk	+	+	+	-	+
	RA0057	2020	Chungcheongbuk	+	+	-	-	+
	RA0058	2020	Chungcheongbuk	+	+	+	-	+
	RA0059	2020	Chungcheongbuk	+	+	+	-	+
	RA0060	2020	Chungcheongbuk	+	+	+	-	+
	RA0061	2020	Chungcheongbuk	+	+	+	-	+
	RA0062	2020	Chungcheongbuk	+	+	+	-	+
	RA0063	2020	Chungcheongbuk	+	+	+	-	+
	RA0064	2020	Chungcheongbuk	+	+	-	-	+
	RA0065	2020	Chungcheongbuk	+	+	+	-	+
	RA0066	2020	Chungcheongbuk	+	+	+	-	+
	RA0067	2020	Chungcheongbuk	+	+	-	-	+

	RA0068	2020	Chungcheongbuk	+	+	+	-	+
	RA0069	2020	Chungcheongbuk	+	+	+	-	+
	RA0070	2020	Chungcheongbuk	+	+	+	-	+
	RA0071	2020	Chungcheongbuk	+	+	+	-	+
	RA0072	2020	Chungcheongbuk	+	+	+	-	+
	RA0073	2020	Chungcheongbuk	+	+	+	-	+
	RA0074	2020	Chungcheongbuk	+	+	+	-	+
	RA0075	2020	Chungcheongbuk	+	+	+	-	+
	RA0076	2020	Chungcheongbuk	+	+	+	-	+
	RA0077	2020	Chungcheongbuk	+	+	+	-	+
	RA0078	2020	Chungcheongbuk	+	+	+	-	+
	RA0079	2020	Chungcheongbuk	+	+	-	-	+
	RA0080	2020	Chungcheongbuk	+	+	+	-	+
	RA0081	2020	Chungcheongbuk	+	+	+	-	+

	RA0082	2020	Chungcheongbuk	+	+	-	-	+
	RA0083	2020	Chungcheongbuk	+	+	-	-	+
	RA0084	2020	Chungcheongbuk	+	+	+	-	+
	RA0085	2020	Chungcheongbuk	+	+	-	-	+
	RA0086	2020	Chungcheongbuk	+	+	-	-	+
	RA0087	2020	Chungcheongbuk	+	+	-	-	+
	RA0088	2020	Chungcheongbuk	+	+	-	-	+
	RA0089	2020	Chungcheongbuk	+	+	+	-	+
	RA0090	2020	Chungcheongbuk	+	+	+	-	+
	RA0091	2020	Chungcheongbuk	+	+	-	-	+
	RA0092	2020	Chungcheongbuk	+	+	-	-	+
	RA0093	2020	Chungcheongbuk	+	+	+	+	+
	RA0094	2020	Chungcheongbuk	+	+	-	-	+
	RA0095	2020	Chungcheongbuk	+	+	+	-	+

Erwinia pyrifoliae	RP0098	2020	Gangwon	-	+	-	+	+
	RP0099	2020	Gangwon	+	+	-	+	+
	RP0100	2020	Gangwon	+	+	+	+	+
	RP0101	2020	Gangwon	+	+	+	+	+
	RP0102	2020	Gangwon	+	+	+	+	+
	RP0103	2020	Gangwon	+	+	+	+	+
	RP0104	2020	Gangwon	+	+	-	+	+
	RP0105	2020	Gangwon	+	+	+	+	+
	RP0106	2020	Gangwon	+	+	+	+	+
	RP0107	2020	Gangwon	+	+	+	+	+
	RP0108	2020	Gangwon	+	+	+	+	+
	RP0109	2020	Gangwon	+	+	+	+	+
	RP0110	2020	Gangwon	-	-	-	-	+
	RP0111	2020	Gyeonggi	+	+	+	+	+

	RP0112	2020	Gyeonggi	+	+	+	+	+
	RP0113	2020	Gyeonggi	+	+	+	+	+
	RP0114	2020	Gyeongsangbuk	+	+	+	+	+
	RP0115	2020	Gyeongsangbuk	+	+	+	+	+
	RP0116	2020	Chungcheongbuk	+	+	+	+	+
	RP0117	2020	Chungcheongbuk	+	+	+	+	+
	RP0118	2020	Chungcheongbuk	+	+	+	+	+
	RP0119	2020	Chungcheongbuk	+	+	+	+	+
	RP0120	2020	Gangwon	+	+	+	+	+
	RP0121	2020	Chungcheongbuk	+	+	+	+	+
Total		***E. amylovora***	**91 (98.91%)**	**90 (97.83%)**	**70 (76.09%)**	**2 (2.17%)**	**91 (98.91%)**	
		E. pyrifoliae	**22 (91.67%)**	**23 (95.83%)**	**19 (79.17%)**	**23 (95.83%)**	**24 (100.00%)**	

Table 2. Morphological characteristics of *Erwinia* phages.

Phage	Capsid (nm)	Tail length (nm)	Virus family
pEp_SNUABM_03	56 ± 2	17 ± 2	*Podoviridae*
pEp_SNUABM_04	55 ± 3	16 ± 2	*Podoviridae*
pEp_SNUABM_11	56 ± 3	18 ± 1	*Podoviridae*
pEp_SNUABM_12	63 ± 2	17 ± 1	*Podoviridae*

Table 3. General genomic features of *Erwinia* phages

Phage	Genome size (bp)	ORFs	GC content (%)	DNA circularity	Accession number
pEp_SNUABM_03	39,879	52	52.13%	circular	MT822284.1
pEp_SNUABM_04	39,649	52	52.19%	circular	MT822285.1
pEp_SNUABM_11	39,626	49	52.10%	circular	MT822287.1
pEp_SNUABM_12	39,980	50	51.19%	circular	MT822288.1

Table 4. Nucleotide identity (%) among the closely related phages. The identity was determined using nucleotide blast algorithm.

	pEp_03	**pEp_04**	**pEp_11**	**L1**	**pEp_12**	**Ninurta**
pEp_03	100	98.6	98.50	74.53	72.13	72.29
pEp_04	-	100	98.18	70.30	72.26	71.83
pEp_11	-	-	100	70.78	72.32	71.83
L1	-	-	-	100	70.85	71.02
pEp_12	-	-	-	-	100	94.66
Ninurta	-	-	-	-	-	100

Table 5. Core genes shared by the *Erwinia* phages analyzed in this study.

pEp_SNUABM_03	pEp_SNUABM_04	pEp_SNUABM_11	pEp_SNUABM_12
hypothetical protein (QOC57603.1)	hypothetical protein (QOC57658.1)	hypothetical protein (QOC57761.1)	hypothetical protein (QOC57812.1)
putative terminase large subunit (QOC57604.1)	putative terminase large subunit (QOC57659.1)	putative terminase large subunit (QOC57762.1)	putative terminase large subunit (QOC57811.1)
hypothetical protein (QOC57605.1)	hypothetical protein (QOC57660.1)	hypothetical protein (QOC57763.1)	hypothetical protein (QOC57810.1)
putative spanin inner membrane subunit (QOC57606.1)	putative spanin inner membrane subunit (QOC57661.1)	putative spanin inner membrane subunit (QOC57764.1)	putative endopeptidase (QOC57809.1)
putative terminase small subunit (QOC57607.1)	putative terminase small subunit (QOC57662.1)	putative terminase small subunit (QOC57765.1)	putative terminase small subunit (QOC57808.1)
putative type II holin (QOC57608.1)	putative type II holin (QOC57663.1)	putative type II holin (QOC57766.1)	putative type II holin (QOC57807.1)

hypothetical protein (QOC57609.1)	hypothetical protein (QOC57664.1)	hypothetical protein (QOC57767.1)	hypothetical protein (QOC57806.1)
putative tail fiber protein (QOC57610.1)	putative tail fiber protein (QOC57665.1)	putative tail fiber protein (QOC57768.1)	putative tail fiber protein (QOC57855.1)
putative internal virion protein D (QOC57611.1)	putative internal virion protein D (QOC57666.1)	putative internal virion protein D (QOC57769.1)	putative internal virion protein D (QOC57854.1)
putative internal virion protein C (QOC57612.1)	putative internal virion protein C (QOC57667.1)	Internal virion protein C (QOC57770.1)	putative internal virion protein C (QOC57853.1)
putative internal virion protein B (QOC57613.1)	putative internal virion protein B (QOC57668.1)	Internal virion protein C (QOC57771.1)	putative tail protein (QOC57852.1)
putative internal core protein (QOC57614.1)	putative internal core protein (QOC57669.1)	putative internal core protein (QOC57772.1)	internal virion protein A (QOC57851.1)
putative tail tubular protein B (QOC57615.1)	putative tail tubular protein B (QOC57670.1)	putative tail tubular protein B (QOC57773.1)	putative tail tubular protein B (QOC57850.1)

putative tail tubular protein A (QOC57616.1)	putative tail tubular protein A (QOC57671.1)	putative tail tubular protein A (QOC57774.1)	putative tail tubular protein A (QOC57849.1)
putative minor capsid protein (QOC57617.1)	putative minor capsid protein (QOC57672.1)	hypothetical protein (QOC57775.1)	
putative major capsid protein (QOC57618.1)	putative major capsid protein (QOC57673.1)	putative major capsid protein (QOC57776.1)	putative major capsid protein (QOC57847.1)
putative capsid assembly scaffolding protein (QOC57619.1)	putative capsid assembly scaffolding protein (QOC57674.1)	putative capsid assembly scaffolding protein (QOC57777.1)	putative capsid assembly scaffolding protein (QOC57846.1)
putative head to tail connecting protein (QOC57620.1)	putative head to tail connecting protein (QOC57675.1)	putative head to tail connecting protein (QOC57778.1)	putative head to tail joining protein (QOC57845.1)
putative virion assembly protein (QOC57621.1)	putative virion assembly protein (QOC57676.1)	putative virion assembly protein (QOC57779.1)	putative tail assembly protein (QOC57844.1)
hypothetical protein (QOC57622.1)	hypothetical protein (QOC57677.1)	hypothetical protein (QOC57780.1)	hypothetical protein (QOC57843.1)

hypothetical protein (QOC57623.1)	hypothetical protein (QOC57678.1)	hypothetical protein (QOC57781.1)	hypothetical protein (QOC57842.1)
hypothetical protein (QOC57624.1)	hypothetical protein (QOC57679.1)	hypothetical protein (QOC57782.1)	hypothetical protein (QOC57841.1)
putative exonuclease (QOC57625.1)	putative exonuclease (QOC57680.1)	putative exonuclease (QOC57783.1)	putative exonuclease (QOC57840.1)
hypothetical protein (QOC57626.1)	hypothetical protein (QOC57681.1)	hypothetical protein (QOC57784.1)	hypothetical protein (QOC57839.1)
hypothetical protein (QOC57627.1)	hypothetical protein (QOC57682.1)	hypothetical protein (QOC57785.1)	putative HNS binding protein (QOC57838.1)
putative HNS binding protein (QOC57628.1)	hypothetical protein (QOC57683.1)	putative HNS binding protein (QOC57786.1)	
putative DNA-directed DNA polymerase (QOC57630.1)	putative DNA-directed DNA polymerase (QOC57685.1)	putative DNA-directed DNA polymerase (QOC57685.1)	putative DNA-directed DNA polymerase (QOC57836.1)

putative inhibitor of toxin/antitoxin system (QOC57631.1)	hypothetical protein (QOC57686.1)	putative inhibitor of toxin/antitoxin system (QOC57788.1)	
hypothetical protein (QOC57632.1)	hypothetical protein (QOC57687.1)	hypothetical protein (QOC57789.1)	
hypothetical protein (QOC57633.1)	hypothetical protein (QOC57688.1)	hypothetical protein (QOC57790.1)	
putative DNA helicase (QOC57634.1)	putative DNA helicase (QOC57689.1)	putative DNA helicase (QOC57791.1)	putative DNA helicase (QOC57833.1)
putative N-acetylmuramoyl-L-alanine amidase (QOC57635.1)	putative N-acetylmuramoyl-L-alanine amidase (QOC57690.1)	putative N-acetylmuramoyl-L-alanine amidase (QOC57792.1)	putative N-acetylmuramoyl-L-alanine amidase (QOC57831.1)
putative endonuclease (QOC57636.1)	putative endonuclease (QOC57691.1)	putative endonuclease (QOC57793.1)	putative endonuclease (QOC57830.1)
putative single-stranded DNA-binding protein (QOC57637.1)	putative single-stranded DNA-binding protein (QOC57692.1)	putative single-stranded DNA-binding protein (QOC57794.1)	putative single-stranded DNA-binding protein (QOC57829.1)

putative host RNA polymerase inhibitor (QOC57638.1)	putative host RNA polymerase inhibitor (QOC57693.1)	putative host RNA polymerase inhibitor (QOC57795.1)	putative bacterial RNA polymerase inhibitor (QOC57827.1)
hypothetical protein (QOC57639.1)	hypothetical protein (QOC57694.1)	hypothetical protein (QOC57796.1)	
hypothetical protein (QOC57640.1)	hypothetical protein (QOC57695.1)	hypothetical protein (QOC57797.1)	
hypothetical protein (QOC57641.1)	hypothetical protein (QOC57696.1)	hypothetical protein (QOC57798.1)	
hypothetical protein (QOC57642.1)	hypothetical protein (QOC57697.1)	hypothetical protein (QOC57799.1)	
hypothetical protein (QOC57643.1)	hypothetical protein (QOC57698.1)	hypothetical protein (QOC57800.1)	
putative DNA ligase (QOC57646.1)	putative DNA ligase (QOC57700.1)	putative DNA ligase (QOC57801.1)	putative DNA ligase (QOC57823.1)

putative host dGTPase inhibitor (QOC57647.1)	putative host dGTPase inhibitor (QOC57701.1)	putative host dGTPase inhibitor (QOC57802.1)	putative inhibitor of dGTPase (QOC57822.1)
hypothetical protein (QOC57648.1)	hypothetical protein (QOC57702.1)	hypothetical protein (QOC57803.1)	
hypothetical protein (QOC57649.1)	hypothetical protein (QOC57703.1)	hypothetical protein (QOC57804.1)	hypothetical protein (QOC57820.1)
putative RNA polymerase (QOC57650.1)	putative RNA polymerase (QOC57704.1)	putative RNA polymerase (QOC57805.1)	putative RNA polymerase (QOC57819.1)
hypothetical protein (QOC57652.1)	hypothetical protein (QOC57655.1)	hypothetical protein (QOC57757.1)	hypothetical protein (QOC57817.1)
putative S-adenosyl-L-methionine hydrolase (QOC57654.1)	putative S-adenosyl-L-methionine hydrolase (QOC57657.1)	putative S-adenosyl-L-methionine hydrolase (QOC57759.1)	putative S-adenosyl-L-methionine hydrolase (QOC57813.1)

Table 6. Functional categories of the predicted open reading frames (ORFs) in *Erwinia* phage pEp_SNUABM_03.

Group	Locus tag	Encoded protein	Related organism	Query cover (%)	Identity (%)
Hypothetical protein	pEp_SNUABM_03_00001	Hypothetical protein	*Erwinia* phage vB_EamP-L1	96	60.42
Structure and packaging	pEp_SNUABM_03_00002	putative terminase large subunit	*Erwinia* phage pEp_SNUABM_09	100	99.83
Hypothetical protein	pEp_SNUABM_03_00003	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	96.57
Lysis	pEp_SNUABM_03_00004	putative spanin inner membrane subunit	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_03_00005	putative terminase small subunit	*Erwinia* phage pEp_SNUABM_09	100	98.85
Lysis	pEp_SNUABM_03_00006	putative type II holin	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_03_00007	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.45
Structure and packaging	pEp_SNUABM_03_00008	putative tail fiber protein	*Erwinia* phage pEp_SNUABM_09	100	99.43
Structure and packaging	pEp_SNUABM_03_00009	putative internal virion protein D	*Erwinia* phage pEp_SNUABM_09	100	99.24
Structure and packaging	pEp_SNUABM_03_00010	putative internal virion protein C	*Erwinia* phage pEp_SNUABM_09	10	100
Structure and packaging	pEp_SNUABM_03_00011	putative internal virion protein B,	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_03_00012	putative internal core protein	*Erwinia* phage pEp_SNUABM_09	100	98.62

Structure and packaging	pEp_SNUABM_03_00013	putative tail tubular protein B	*Erwinia* phage pEp_SNUABM_09	100	99.62
Structure and packaging	pEp_SNUABM_03_00014	putative tail tubular protein A	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_03_00015	putative minor capsid protein	*Erwinia* phage pEp_SNUABM_09	100	95
Structure and packaging	pEp_SNUABM_03_00016	putative major capsid protein	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_03_00017	putative capsid assembly scaffolding protein	*Erwinia* phage pEp_SNUABM_09	100	99.36
Structure and packaging	pEp_SNUABM_03_00018	putative head to tail connecting protein	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_03_00019	putative virion assembly protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_03_00020	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_03_00021	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.77
Hypothetical protein	pEp_SNUABM_03_00022	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	97.5
Nucleotide regulation	pEp_SNUABM_03_00023	putative exonuclease	*Erwinia* phage pEp_SNUABM_09	100	99.67
Hypothetical protein	pEp_SNUABM_03_00024	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_03_00025	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Nucleotide regulation	pEp_SNUABM_03_00026	putative HNS binding protein	*Erwinia* phage pEp_SNUABM_09	100	97.8

Hypothetical protein	pEp_SNUABM_03_00027	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Nucleotide regulation	pEp_SNUABM_03_00028	putative DNA-directed DNA polymerase	*Erwinia* phage pEp_SNUABM_09	100	99.72
Additional function	pEp_SNUABM_03_00029	putative inhibitor of toxin/antitoxin system	*Erwinia* phage pEp_SNUABM_09	100	89.47
Hypothetical protein	pEp_SNUABM_03_00030	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_03_00031	Hypothetical protein	N/A[a]	N/A	N/A
Nucleotide regulation	pEp_SNUABM_03_00032	putative DNA helicase	*Erwinia* phage pEp_SNUABM_09	89	100
Lysis	pEp_SNUABM_03_00033	putative N-acetylmuramoyl-L-alanine amidase	*Erwinia* phage pEp_SNUABM_09	100	98.68
Nucleotide regulation	pEp_SNUABM_03_00034	putative endonuclease	*Erwinia* phage pEp_SNUABM_09	100	100
Nucleotide regulation	pEp_SNUABM_03_00035	putative single-stranded DNA-binding protein	*Erwinia* phage pEp_SNUABM_09	100	99.13
Additional function	pEp_SNUABM_03_00036	putative host RNA polymerase inhibitor	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_03_00037	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_03_00038	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	98	85.88

Hypothetical protein	pEp_SNUABM_03_00039	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_03_00040	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_03_00041	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.21
Hypothetical protein	pEp_SNUABM_03_00042	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	88.52
Hypothetical protein	pEp_SNUABM_03_00043	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.36
Nucleotide regulation	pEp_SNUABM_03_00044	putative DNA ligase	*Erwinia* phage pEp_SNUABM_09	100	93.24
Nucleotide regulation	pEp_SNUABM_03_00045	putative host dGTPase inhibitor	*Erwinia* phage pEp_SNUABM_09	62	98.08
Hypothetical protein	pEp_SNUABM_03_00046	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_03_00047	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	96.37
Nucleotide regulation	pEp_SNUABM_03_00048	putative RNA polymerase	*Erwinia* phage pEp_SNUABM_09	100	100
Nucleotide regulation	pEp_SNUABM_03_00049	putative protein kinase	*Dickeya* phage Ninurta	70	52.87
Hypothetical protein	pEp_SNUABM_03_00050	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	79	100
Hypothetical protein	pEp_SNUABM_03_00051	Hypothetical protein	N/A	N/A	N/A
Nucleotide regulation	pEp_SNUABM_03_00052	putative S-adenosyl-L-methionine hydrolase	*Erwinia* phage pEp_SNUABM_09	95	95.12

[a]N/A, Not available.

Table 7. Functional categories of the predicted open reading frames (ORFs) in *Erwinia* phage pEp_SNUABM_04.

Group	Locus tag	Encoded protein	Related organism	Query cover (%)	Identity (%)
Hypothetical protein	pEp_SNUABM_04_00001	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	78	98.04
Hypothetical protein	pEp_SNUABM_04_00002	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	97	100
Nucleotide regulation	pEp_SNUABM_04_00003	putative S-adenosyl-L-methionine hydrolase	*Erwinia* phage pEp_SNUABM_09	98	99.35
Hypothetical protein	pEp_SNUABM_04_00004	Hypothetical protein	*Yersinia* phage Berlin	94	54.17
Structure and packaging	pEp_SNUABM_04_00005	putative terminase large subunit	*Erwinia* phage pEp_SNUABM_09	99	99.83
Hypothetical protein	pEp_SNUABM_04_00006	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	95.59
Lysis	pEp_SNUABM_04_00007	putative spanin inner membrane subunit	*Erwinia* phage pEp_SNUABM_09	100	99.32
Nucleotide regulation	pEp_SNUABM_04_00008	putative terminase small subunit	*Erwinia* phage pEp_SNUABM_09	100	100
Lysis	pEp_SNUABM_04_00009	putative type II holin	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_04_00010	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	97.67
Structure and packaging	pEp_SNUABM_04_00011	putative tail fiber protein	*Erwinia* phage pEp_SNUABM_09	100	99.43
Structure and packaging	pEp_SNUABM_04_00012	putative internal virion protein D	*Erwinia* phage pEp_SNUABM_09	100	99.24
Structure and packaging	pEp_SNUABM_04_00013	putative internal virion protein C	*Erwinia* phage pEp_SNUABM_09	100	99.87

Structure and packaging	pEp_SNUABM_04_00014	putative internal virion protein B	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_04_00015	putative internal core protein	*Erwinia* phage pEp_SNUABM_09	100	97.93
Structure and packaging	pEp_SNUABM_04_00016	putative tail tubular protein B	*Erwinia* phage pEp_SNUABM_09	100	99.75
Structure and packaging	pEp_SNUABM_04_00017	putative tail tubular protein A	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_04_00018	putative minor capsid protein	*Erwinia* phage pEp_SNUABM_09	100	97.5
Structure and packaging	pEp_SNUABM_04_00019	putative major capsid protein	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_04_00020	putative capsid assembly scaffolding protein	*Erwinia* phage pEp_SNUABM_09	100	99.36
Structure and packaging	pEp_SNUABM_04_00021	putative head to tail connecting protein	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_04_00022	putative virion assembly protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_04_00023	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_04_00024	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.77
Hypothetical protein	pEp_SNUABM_04_00025	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	97.5
Nucleotide regulation	pEp_SNUABM_04_00026	putative exonuclease	*Erwinia* phage pEp_SNUABM_09	100	99.34
Hypothetical protein	pEp_SNUABM_04_00027	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.98

Hypothetical protein	pEp_SNUABM_04_00028	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_04_00029	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.9
Hypothetical protein	pEp_SNUABM_04_00030	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.11
Nucleotide regulation	pEp_SNUABM_04_00031	putative DNA-directed DNA polymerase	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_04_00032	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	89.47
Hypothetical protein	pEp_SNUABM_04_00033	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	97.14
Hypothetical protein	pEp_SNUABM_04_00034	Hypothetical protein	N/A[a]	N/A	N/A
Nucleotide regulation	pEp_SNUABM_04_00035	putative DNA helicase	*Erwinia* phage pEp_SNUABM_09	89	100
Lysis	pEp_SNUABM_04_00036	putative N-acetylmuramoyl-L-alanine amidase	*Erwinia* phage pEp_SNUABM_09	100	98.68
Nucleotide regulation	pEp_SNUABM_04_00037	putative endonuclease	*Erwinia* phage pEp_SNUABM_09	100	100
Nucleotide regulation	pEp_SNUABM_04_00038	putative single-stranded DNA-binding protein	*Erwinia* phage pEp_SNUABM_09	100	99.13
Additional function	pEp_SNUABM_04_00039	putative host RNA polymerase inhibitor	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_04_00040	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_04_00041	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	99	61.78
Hypothetical protein	pEp_SNUABM_04_00042	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.88
Hypothetical protein	pEp_SNUABM_04_00043	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_04_00044	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.21

Hypothetical protein	pEp_SNUABM_04_00045	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	88.52
Nucleotide regulation	pEp_SNUABM_04_00046	putative DNA ligase	*Erwinia* phage pEp_SNUABM_09	100	91.6
Additional function	pEp_SNUABM_04_00047	putative host dGTPase inhibitor	*Erwinia* phage pEp_SNUABM_09	62	98.08
Hypothetical protein	pEp_SNUABM_04_00048	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_04_00049	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.45
Structure and packaging	pEp_SNUABM_04_00050	putative RNA polymerase	*Erwinia* phage pEp_SNUABM_09	100	100

[a]N/A, Not available

Table 8. Functional categories of the predicted open reading frames (ORFs) in *Erwinia* phage pEp_SNUABM_11.

Group	Locus tag	Encoded protein	Related organism	Query cover (%)	Identity (%)
Hypothetical protein	pEp_SNUABM_11_00001	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	79	100
Hypothetical protein	pEp_SNUABM_11_00002	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	97.73
Nucleotide regulation	pEp_SNUABM_11_00003	putative S-adenosyl-L-methionine hydrolase	*Erwinia* phage pEp_SNUABM_09	99	98.05
Hypothetical protein	pEp_SNUABM_11_00004	Hypothetical protein	*Yersinia* phage Berlin	45	59.38
Hypothetical protein	pEp_SNUABM_11_00005	Hypothetical protein	*Erwinia* phage vB_EamP-L1	96	60.42
Structure and packaging	pEp_SNUABM_11_00006	putative terminase large subunit	*Erwinia* phage pEp_SNUABM_09	100	99.83
Hypothetical protein	pEp_SNUABM_11_00007	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	94.12
Lysis	pEp_SNUABM_11_00008	putative spanin inner membrane subunit	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_11_00009	putative terminase small subunit	*Erwinia* phage pEp_SNUABM_09	100	100
Lysis	pEp_SNUABM_11_00010	putative type II holin	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_11_00011	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.45
Structure and packaging	pEp_SNUABM_11_00012	putative tail fiber protein	*Erwinia* phage pEp_SNUABM_09	100	99.06
Structure and packaging	pEp_SNUABM_11_00013	putative internal virion protein D	*Erwinia* phage pEp_SNUABM_09	100	99.01

Structure and packaging	pEp_SNUABM_11_00014	Internal virion protein C	*Erwinia* phage pEp_SNUABM_09	100	99.87
Structure and packaging	pEp_SNUABM_11_00015	Internal virion protein C	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_11_00016	putative internal core protein	*Erwinia* phage pEp_SNUABM_09	100	97.24
Structure and packaging	pEp_SNUABM_11_00017	putative tail tubular protein B	*Erwinia* phage pEp_SNUABM_09	100	99.87
Structure and packaging	pEp_SNUABM_11_00018	putative tail tubular protein A	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_11_00019	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	95
Structure and packaging	pEp_SNUABM_11_00020	putative major capsid protein	*Erwinia* phage pEp_SNUABM_09	100	99.71
Structure and packaging	pEp_SNUABM_11_00021	putative capsid assembly scaffolding protein	*Erwinia* phage pEp_SNUABM_09	100	99.36
Structure and packaging	pEp_SNUABM_11_00022	putative head to tail connecting protein	*Erwinia* phage pEp_SNUABM_09	100	100
Structure and packaging	pEp_SNUABM_11_00023	putative virion assembly protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_11_00024	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_11_00025	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.77
Hypothetical protein	pEp_SNUABM_11_00026	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	95
Nucleotide regulation	pEp_SNUABM_11_00027	putative exonuclease	*Erwinia* phage pEp_SNUABM_09	100	99.67

Hypothetical protein	pEp_SNUABM_11_00028	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_11_00029	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Nucleotide regulation	pEp_SNUABM_11_00030	putative HNS binding protein	*Erwinia* phage pEp_SNUABM_09	100	98.9
Nucleotide regulation	pEp_SNUABM_11_00031	putative DNA-directed DNA polymerase	*Erwinia* phage pEp_SNUABM_09	100	99.86
Additional function	pEp_SNUABM_11_00032	putative inhibitor of toxin/antitoxin system	*Erwinia* phage pEp_SNUABM_09	100	90.53
Hypothetical protein	pEp_SNUABM_11_00033	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_11_00034	Hypothetical protein	N/A[a]	N/A	N/A
Nucleotide regulation	pEp_SNUABM_11_00035	putative DNA helicase	*Erwinia* phage pEp_SNUABM_09	89	99.8
Lysis	pEp_SNUABM_11_00036	putative N-acetylmuramoyl-L-alanine amidase	*Erwinia* phage pEp_SNUABM_09	100	99.34
Nucleotide regulation	pEp_SNUABM_11_00037	putative endonuclease	*Erwinia* phage pEp_SNUABM_09	100	100
Nucleotide regulation	pEp_SNUABM_11_00038	putative single-stranded DNA-binding protein	*Erwinia* phage pEp_SNUABM_09	100	98.69
Additional function	pEp_SNUABM_11_00039	putative host RNA polymerase inhibitor	*Erwinia* phage pEp_SNUABM_09	100	100
hypothetical protein	pEp_SNUABM_11_00040	hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_11_00041	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	82.99
Hypothetical protein	pEp_SNUABM_11_00042	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	96.7
Hypothetical protein	pEp_SNUABM_11_00043	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_11_00044	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	98.21

Nucleotide regulation	pEp_SNUABM_11_00045	putative DNA ligase	*Erwinia* phage pEp_SNUABM_09	100	86.53
Additional function	pEp_SNUABM_11_00046	putative host dGTPase inhibitor	*Erwinia* phage pEp_SNUABM_09	62	98.08
Hypothetical protein	pEp_SNUABM_11_00047	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	100
Hypothetical protein	pEp_SNUABM_11_00048	Hypothetical protein	*Erwinia* phage pEp_SNUABM_09	100	95.34
Nucleotide regulation	pEp_SNUABM_11_00049	putative RNA polymerase	*Erwinia* phage pEp_SNUABM_09	100	100

[a]N/A, Not available.

Table 9. Functional categories of the predicted open reading frames (ORFs) in *Erwinia* phage pEp_SNUABM_12.

Group	Locus tag	Encoded protein	Related organism	Query cover (%)	Identity (%)
Hypothetical protein	pEp_SNUABM_12_00001	Hypothetical protein	*Klebsiella* phage vB_KpnP_Sibilus	100	98.41
Lysis	pEp_SNUABM_12_00002	putative type II holin	*Dickeya* phage Ninurta	100	100
Structure and packaging	pEp_SNUABM_12_00003	putative terminase small subunit	*Dickeya* phage Ninurta	100	98.85
Lysis	pEp_SNUABM_12_00004	putative endopeptidase	*Klebsiella* phage vB_KpnP_Sibilus	100	99.38
Hypothetical protein	pEp_SNUABM_12_00005	Hypothetical protein	*Klebsiella* phage vB_KpnP_Sibilus	100	92.2
Structure and packaging	pEp_SNUABM_12_00006	putative terminase large subunit	*Klebsiella* phage vB_KpnP_NahiliMali	100	99.66
Hypothetical protein	pEp_SNUABM_12_00007	Hypothetical protein	*Klebsiella* phage vB_KpnP_NahiliMali	100	98.08
Nucleotide regulation	pEp_SNUABM_12_00008	putative S-adenosyl-L-methionine hydrolase	*Klebsiella* phage vB_KpnP_Sibilus	100	100
Hypothetical protein	pEp_SNUABM_12_00009	Hypothetical protein	*Klebsiella* phage vB_KpnP_Sibilus	100	97.96
Hypothetical protein	pEp_SNUABM_12_00010	Hypothetical protein	*Dickeya* phage vB_DsoP_JA10	97	95.65
Hypothetical protein	pEp_SNUABM_12_00011	Hypothetical protein	*Klebsiella* phage vB_KpnP_Sibilus	100	100
Hypothetical protein	pEp_SNUABM_12_00012	Hypothetical protein	*Klebsiella* phage vB_KpnP_Sibilus	100	100

Nucleotide regulation	pEp_SNUABM_12_00013	putative protein kinase	*Dickeya* phage vB_DsoP_JA10	100	83.38
Nucleotide regulation	pEp_SNUABM_12_00014	putative RNA polymerase	*Dickeya* phage vB_DsoP_JA10	100	99.32
Hypothetical protein	pEp_SNUABM_12_00015	Hypothetical protein	*Dickeya* phage Ninurta	100	98.77
Hypothetical protein	pEp_SNUABM_12_00016	Hypothetical protein	*Klebsiella* phage vB_KpnP_Sibilus	100	98.28
Additional function	pEp_SNUABM_12_00017	putative inhibitor of dGTPase	*Dickeya* phage vB_DsoP_JA10	100	79.31
Nucleotide regulation	pEp_SNUABM_12_00018	putative DNA ligase	*Dickeya* phage vB_DsoP_JA10	99	98.54
Hypothetical protein	pEp_SNUABM_12_00019	Hypothetical protein	*Klebsiella* phage vB_KpnP_Sibilus	100	100
Hypothetical protein	pEp_SNUABM_12_00020	Hypothetical protein	*Dickeya* phage vB_DsoP_JA10	100	98.82
Hypothetical protein	pEp_SNUABM_12_00021	Hypothetical protein	*Dickeya* phage Ninurta	100	98.58
Additional function	pEp_SNUABM_12_00022	putative bacterial RNA polymerase inhibitor	*Klebsiella* phage vB_KpnP_NahiliMali	100	100
Hypothetical protein	pEp_SNUABM_12_00023	Hypothetical protein	*Dickeya* phage vB_DsoP_JA10	100	99.18
Nucleotide regulation	pEp_SNUABM_12_00024	putative single-stranded DNA-binding protein	*Klebsiella* phage vB_KpnP_Sibilus	100	99.57
Nucleotide regulation	pEp_SNUABM_12_00025	putative endonuclease	*Klebsiella* phage vB_KpnP_Sibilus	100	100

Lysis	pEp_SNUABM_12_00026	putative N-acetylmuramoyl-L-alanine amidase	*Klebsiella* phage vB_KpnP_Sibilus	100	98.68
Nucleotide regulation	pEp_SNUABM_12_00027	putative nucleotidyltransferase	*Klebsiella* phage vB_KpnP_Sibilus	100	97.01
Nucleotide regulation	pEp_SNUABM_12_00028	putative DNA helicase	*Klebsiella* phage vB_KpnP_Sibilus	100	99.3
Hypothetical protein	pEp_SNUABM_12_00029	Hypothetical protein	*Dickeya* phage vB_DsoP_JA10	100	93.51
Hypothetical protein	pEp_SNUABM_12_00030	Hypothetical protein	*Klebsiella* phage vB_KpnP_Sibilus	100	100
Nucleotide regulation	pEp_SNUABM_12_00031	putative DNA-directed DNA polymerase	*Dickeya* phage Ninurta	100	99.71
Nucleotide regulation	pEp_SNUABM_12_00032	putative HNS binding protein	*Klebsiella* phage vB_KpnP_Sibilus	100	96.15
Nucleotide regulation	pEp_SNUABM_12_00033	putative HNS binding protein	*Klebsiella* phage vB_KpnP_NahiliMali	100	100
Hypothetical protein	pEp_SNUABM_12_00034	Hypothetical protein	*Klebsiella* phage vB_KpnP_Sibilus	100	98.02
Nucleotide regulation	pEp_SNUABM_12_00035	putative exonuclease	*Klebsiella* phage vB_KpnP_Sibilus	100	97.43
Hypothetical protein	pEp_SNUABM_12_00036	Hypothetical protein	N/A[a]	N/A	N/A
Hypothetical protein	pEp_SNUABM_12_00037	Hypothetical protein	*Dickeya* phage Ninurta	100	98.81
Hypothetical protein	pEp_SNUABM_12_00038	Hypothetical protein	*Klebsiella* phage vB_KpnP_Sibilus	100	98.98
Structure and packaging	pEp_SNUABM_12_00039	putative tail assembly protein	*Klebsiella* phage vB_KpnP_Sibilus	100	98.08

Structure and packaging	pEp_SNUABM_12_00040	putative head to tail joining protein	*Dickeya* phage vB_DsoP_JA10	100	100
Structure and packaging	pEp_SNUABM_12_00041	putative capsid assembly scaffolding protein	*Klebsiella* phage vB_KpnP_Sibilus	100	98.6
Structure and packaging	pEp_SNUABM_12_00042	putative major capsid protein	*Dickeya* phage Ninurta	100	99.71
Structure and packaging	pEp_SNUABM_12_00043	putative minor capsid protein	*Klebsiella* phage vB_KpnP_Sibilus	100	98.68
Structure and packaging	pEp_SNUABM_12_00044	putative tail tubular protein A	*Klebsiella* phage vB_KpnP_Sibilus	100	98.97
Structure and packaging	pEp_SNUABM_12_00045	putative tail tubular protein B	*Klebsiella* phage vB_KpnP_Sibilus	100	99.37
Structure and packaging	pEp_SNUABM_12_00046	internal virion protein A	*Klebsiella* phage vB_KpnP_Sibilus	100	99.3
Structure and packaging	pEp_SNUABM_12_00047	putative tail protein	*Klebsiella* phage vB_KpnP_NahiliMali	100	98.48
Structure and packaging	pEp_SNUABM_12_00048	putative internal virion protein C	*Klebsiella* phage vB_KpnP_Sibilus	100	99.47
Structure and packaging	pEp_SNUABM_12_00049	putative internal virion protein D	*Klebsiella* phage vB_KpnP_Sibilus	100	99.17
Structure and packaging	pEp_SNUABM_12_00050	putative tail fiber protein	*Klebsiella* phage vB_KpnP_Sibilus	100	96.38

[a]N/A, Not available.

Table 10. Host range analysis of individual and combined *Erwinia* phages, alone and as and the combined cocktail.

Bacteria	**pEp_SNUABM_03**	**pEp_SNUABM_04**	**pEp_SNUABM_11**	**pEp_SNUABM_12**	**Cocktail phage**
E. amylovora	98.91% (91/92)	97.83% (90/92)	76.09% (70/92)	2.17% (2/92)	98.91% (91/92)
E. pyrifoliae	92.00% (22/24)	95.83% (23/24)	79.17% (19/24)	95.83% (23/24)	100.00% (24/24)

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Summary

We isolated four phages, pEp_SNUABM_03, 04, 11, and 12, effective against both *E. amylovora* and *E. pyrifoliae* pathogens, and investigated their biological and genomic properties. Phages showed infectivity to both pathogens of *Erwinia* and was able to control these pathogens effectively over a long period of time. The cocktail treatment has the advantage of broadening the host spectrum as well as inducing synergistic effects. In addition, the stability and safety of phages for use as biocontrol agents were verified. Taken together, combining several phages that have distinct infection strategies and administering the cocktail phage suspension would be a remarkable way to control both *Erwinia amylovora*- and *E. pyrifoliae*- caused blight disease in South Korea. However, intensive verifications such as combined treatment with conventional agents, antibacterial efficacy in planta, and field tests, should be performed in further studies.

Abstract in Korean

에르위니아 아밀로보라와 에르위니아 피리폴리애에 의해 발생하는 장미과 식물의 마름병에 대한 파지 기반 생물학적 방제법 개발

조 수 진

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수의학과 수의병인생물학 및 예방수의학 전공

(지도교수: 박 세 창, D.V.M, Ph.D.)

최근 국내에서는 에르위니아 아밀로보라 (*Erwinia amylovora*)와 에르위니아 피리폴리애(*Erwinia pyrifoliae*)라는 서로 구별되지 않는 두 종의 에르위니아가 발생하면서 병해충이 발생해 큰 우려를 낳고 있습니다. 항생제 방제를 중심으로 한 엄격한 관리 프로토콜이 있지만, 발생 지역과 건수가 증가하고 있습니다. 본 연구에서는 에르위니아 아밀로보라와 에르위니아 피리폴리아애를 모두 감염시키는 박테리오파지 4종

(pEp_SNUABM_03, 04, 11, 12)을 분리하여 국내 에르위니아 유래 마름병에 대한 항균제로서의 가능성을 평가했습니다. 형태학적 분석 결과 모든 파지는 *Podovirus*와 유사한 캡시드를 가지고 있는 것으로 나타났습니다. 파지 칵테일은 에르위니아 아밀로보라의 98.91%와 에르위니아 피리폴리애 균주의 100%를 감염시키는 광범위한 감염력을 보여주었습니다. 항균 효과는 에르위니아 아밀로보라에 대한 장기간 칵테일 처리 후 관찰되었고, 에르위니아 피리폴리애에 대한 단기 및 장기 처리 모두에서 관찰되었습니다. 게놈 분석 결과 파지는 항생제 내성이나 독성 유전자와 같은 유해한 유전자를 암호화하지 않는 것으로 확인되었습니다. 모든 파지는 일반적인 과수원 조건에서 안정적이었습니다. 종합적으로, 우리는 에르위니아 아밀로보라와 에르위니아 피리폴리애를 모두 표적으로 하는 생물학적 방제제로서 파지의 잠재력에 대한 기초 데이터를 제공했습니다.

핵심어: 박테리오파지; 에르위니아 마름병; 이과류; 파지 칵테일; 농업

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List of articles

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1. **Su Jin Jo**, Sang Guen Kim, Young Min Lee, Sib Sankar Giri, Jeong Woo. Kang, Sung Bin Lee, Won Joon Jung, Mae Hyun Hwang, Jaehong Park, Chi Cheng, Eunjung Roh, Se Chang Park*. The evaluation of the antimicrobial potential and characterization of novel T7-like *Erwinia* bacteriophages. *Biology*. *12*(2), 180.

2. **Su Jin Jo**, Sang Guen Kim, Jungkum Park, Young Min Lee, Sib Sankar Giri, Sung Bin Lee, Won Joon Jung, Mae Hyun Hwang, Jae Hong Park, Eunjung Roh, and Se Chang Park*. Optimizing the Formulation of *Erwinia* Bacteriophages for Improved UV Stability and Absorption in Plants. *Heliyon* in submission.

3. Sib Sankar Giri, Sang Guen Kim, Kang Jeong Woo, Won Joon Jung, Sung Bin Lee, Young Min Lee, **Su Jin Jo**, Mae Hyun Hwang, Jae Hong Park, Ji Hyung Kim, Sukumaran V, Se Chang Park*. Effects of *Bougainvillea glabra* leaf on growth, skin mucosal immune responses, and disease resistance in common carp *Cyprinus carpio*. *Fish and Shellfish Immunology*. *132*, 108514.

4. Sib Sankar Giri, Sang Guen Kim, Won Joon Jung, Sung Bin Lee, Young Min Lee, **Su Jin Jo**, Mae Hyun Hwang, Jae Hong Park, Ji Hyung Kim,

Subrata Saha, Venkatachalam Sukumaran, Se Chang Park*. Dietary Syzygium cumini leaf extract influences growth performance, immunological responses and gene expression in pathogen-challenged Cyprinus carpio. *Fish and Shellfish Immunology*. 2023(138) : 108830.

2022 Published

1. Sang Wha Kim, Hyoun Joong Kim, Sang Guen Kim, Jun Kwon, Sung Bin Lee, Won Joon Jung, Young Min Lee, **Su Jin Jo**, Sib Sankar Giri, Seok Hyun Yoon, Seon Ho Kim, Chan Mo Kim, Cheng Chi, Se Chang Park*. Bactericidal efficacy of non-thermal plasma activation against *Aeromonas hydrophila* and immunological responses of koi (*Cyprinus carpio haematopterus*). *Fish and Shellfish Immunology. 121*, 197-204.
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3. Jin Woo Jun, Jeong Woo Kang, Sib Sankar Giri, Sang Wha Kim, Sang Guen Kim, Jun Kwon, Sung Bin Lee, Won Joon Jung, Young Min Lee, **Su Jin Jo**, Se Chang Park*. Preventive effect of starch hydrogel-based oral vaccine against *Aeromonas salmonicida* infection in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture. 555*, 738202.
4. Sib Sankar Giri, Sang Guen Kim, Kang Jeong Woo, Won Joon Jung,

Sung Bin Lee, Young Min Lee, **Su Jin Jo**, Ji Hyung Kim, Se Chang Park*. Impact of dandelion polysaccharides on growth and immunity response in common carp *Cyprinus carpio*. *Fish and Shellfish Immunology*. *128*, 371-379.

5. Sang Guen Kim, Sib Sankar Giri, **Su Jin Jo**, Jeong Woo Kang, Sung Bin Lee, Won Joon Jung, Young Min Lee, Hee Jin Kim, Ji Hyung Kim, Se Chang Park*. Prolongation of Fate of Bacteriophages In Vivo by Polylactic-Co-Glycolic-Acid/Alginate-Composite Encapsulation. *Antibiotics*. *11*(9), 1264.
6. Sang-Guen Kim, Sung-Bin Lee, **Su-Jin Jo**, Kevin Cho, Jung-Kum Park, Jun Kwon, Sib Sankar Giri, Sang-Wha Kim, Jeong-Woo Kang, Won-Joon Jung, Young-Min Lee, Eunjung Roh, Se Chang Park*. Phage Cocktail in Combination with Kasugamycin as a Potential Treatment for Fire Blight Caused by *Erwinia amylovora*. *Antibiotics*. *11*(11), 1566.

List of conferences

2022

1. **Su Jin Jo**, Sang Guen Kim, Se Chang Park, International Meeting of the Microbiological Society of Korea (MSK), Republic of Korea, 30th October- 1th November, 2022

감사의 글

길게만 느껴졌던 학위 과정 동안 많은 분들의 도움으로 졸업을 할 수 있었습니다. 모든 분들의 변함없는 지지와 격려에 지금의 제가 있을 수 있다고 생각합니다. 덕분에 지치지 않을 수 있는 힘과 끝없는 동기부여가 되었습니다. 학위 과정 동안 뛰어난 분들로부터 많은 것들을 배우고 지도를 받으며 연구를 할 수 있는 기회를 가지게 된 것은 큰 영광이었습니다. 연구에 진심을 다하는 분들의 모습과 열정에 저도 즐거운 연구실 생활을 하게 되었습니다. 그 시간들 속에서 저에게 항상 많은 힘이 되어주셨던 분들께 이 글을 통해 감사한 마음의 일부를 전해드리고자 합니다.

가장 먼저 대학원 과정 동안 항상 부족했던 저를 끝까지 지도해주시고 큰 도움을 주신 박세창 교수님께 깊은 감사의 말씀을 드립니다. 아낌없는 지도와 따뜻한 가르침 덕분에 연구자로서 길을 잃지 않고 나아갈 수 있었습니다.

그리고 저의 성장과 발전을 항상 응원해주신 이승준 교수님께도 감사의 마음을 전해드리고 싶습니다. 저 자신조차도 의심스러울 때 언제나 밝은 미소로 맞아주시고, 믿어주시고 응원해주신 덕분에 저에게는 정말 큰 힘이 되었습니다.

너무나도 부족했던 저의 멘토가 되어주신 김상근 박사님, 누구도 쉽게 해줄 수 없는 부분까지도 바쁘신 시간 와중에 꼼꼼히 가르쳐주신 덕분에 여기까지 올 수 있었습니다. 한결 같은 연구자의 모습으로 존경스러웠고, 힘든 순간마다 포기하지 않을 수 있게

끝까지 이끌어주셔서 감사한 마음을 꼭 전하고 싶습니다.
부족했던 저임에도 불구하고 바쁘신 와중에 학위 논문의 심사를 맡아주신 심사위원분들께도 감사의 말씀을 전하고 싶습니다. 심사위원장을 맡아 많은 조언을 아끼지 않고 해주신 덕분에, 새로운 부분도 알아가고 부족한 부분을 채울 수 있는 기회를 주신 윤화영 교수님, 세심하게 가르쳐주시고 언제나 저희에게 도움을 주시는 전진우 교수님께 진심으로 감사하다고 말씀을 드리고 싶습니다. 소중한 심사위원 분들의 지도를 바탕으로 앞으로도 끝없이 발전하는 연구자가 되기 위해 노력하겠습니다.
그리고 농촌진흥청에서 많은 지원과 격려를 해주신 이용환 박사님, 노은정 박사님, 여수환 박사님께도 감사하다는 말씀을 전하고 싶습니다. 따뜻한 미소로 맞아주시고 부족하지 않게 아끼지 않고 지원해주신 덕분에 연구를 끝까지 마칠 수 있게 해주셨습니다. 잊지 않고 항상 감사한 마음을 지니고 있겠습니다.
학위 과정 동안 실험실에서 긴 시간을 함께 보낸 선생님들께도 감사하다고 전하고 싶습니다. 먼저, 김상화 박사님께서 저를 응원해주시고 고민도 들어주시며 다정하게 대해주신 덕분에 많은 순간들을 이겨낼 수 있었습니다. 그리고 언제나 즐겁고 재미있는 모습을 보여주시고 많은 조언도 해주시는 권준 박사님께도 고마움을 전하고 싶습니다. 오랜 시간 실험실에서 한결 같은 모습으로 도와주시고 큰 힘이 되어주신 강정우 박사님, Sib Sankar Giri 박사님, 이성빈, 정원준, 이영민, 박재홍, 황매현 선생님께도 고마웠다는 말을 전하고 싶습니다. 힘든 순간에도 고민도 나누고 조언도 해주며

서로에게 힘이 되어 끝까지 나아갈 수 있었습니다.

저에게 아낌없는 지원을 해주신 가족들에게도 깊은 감사를 전합니다. 지칠 때마다 제가 기댈 수 있게 따뜻한 품을 내어주셔서 감사했습니다.

많은 분들의 도움을 받아 온 만큼 저도 도움을 줄 수 있는 사람으로 감사한 마음을 잊지 않고 나아가겠습니다. 감사합니다.

2023 년 8 월

조 수 진