



Research Article

# Characterization of a lytic phage KFS-EC3 infecting multiple foodborne pathogens

Su-Hyeon Kim<sup>1,2</sup>, Ye-Rim Park<sup>1,2</sup>, Hyeju Jung<sup>1,2</sup>, Mi-Kyung Park<sup>1,2\*</sup>

<sup>1</sup>School of Food Science and Biotechnology, Kyungpook National University, Daegu 41566, Korea

<sup>2</sup>Food and Bio-Industry Research Institute, Kyungpook National University, Daegu 41566, Korea

**Abstract** The purpose of this study was to characterize lytic and genomic properties of phage KFS-EC3 used as a biocontrol agent. The morphology of KFS-EC3 was observed by transmission electron microscopy (TEM). The polyvalent bactericidal activity of KFS-EC3 was determined against various host bacteria by enumerating bacterial number after phage treatment at multiplicities of infection (MOIs) of 0.001, 0.1, and 1.0 for 4 h. Finally, whole-genome sequencing was performed and annotated to identify its lytic property, safety, and novelty. TEM observation revealed that KFS-EC3 had morphology of myophage with an icosahedral head and contractile tail. In addition, KFS-EC3 could reduce *E. coli*, *Salmonella*, *Shigella sonnei*, and their bacterial cocktail by ~3 log CFU/mL, even at the lowest MOI of 0.001. The genomic analyses revealed that KFS-EC3 consisted of 167,440 bp and 151 functional genes, without any genes related to antibiotic resistance, virulence, allergenicity, and lysogenicity. Among the genes involved in host cell lysis, endolysin, holin, and a receptor binding protein were identified. Furthermore, KFS-EC3 was classified into a new phage of the *Teqatrovirus* genus in the *Myoviridae* family. In conclusion, lytic and safe phage KFS-EC3 with a broad host range can be used as a biocontrol agent to control foodborne pathogens.



OPEN ACCESS

**Citation:** Kim SH, Park YR, Jung H, Park MK. Characterization of a lytic phage KFS-EC3 infecting multiple foodborne pathogens. Korean J Food Preserv, 29(7), 1022-1034 (2022)

**Received:** November 09, 2022

**Revised:** November 24, 2022

**Accepted:** November 26, 2022

**\*Corresponding author**

Mi-Kyung Park  
Tel: +82-53-950-5776  
E-mail: parkmik@knu.ac.kr

Copyright © 2022 The Korean Society of Food Preservation. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Keywords** lytic phage, genomic analysis, broad host range, safety, novelty

## 1. Introduction

The Centers for Disease Control (CDC) reported that *Escherichia coli*, *Salmonella*, and *Shigella* were considered major foodborne pathogens that caused 50,390 outbreaks with 8,010 hospitalizations during 2009–2020 in the USA (CDC, 2022). These foodborne pathogens are commonly prevalent in foods such as ground beef, dairy products, chicken, and eggs (Duc et al., 2020; Rangel et al., 2005; Shahin and Bouzari, 2018), and recently in fresh produce (Bai et al., 2019; Ruengvisesh et al., 2019). Several physical and chemical methods have been introduced and utilized in fresh produce to control foodborne pathogens using a thermal treatment (Schweiggert et al., 2007), irradiation (Molins et al., 2001), chlorine treatment (Petri et al., 2015), and ozone treatment (Chaidez et al., 2007).

The thermal treatment, popularly used in meat and meat products, is limited when employed in fresh produce due to the loss of nutrients and freshness (Fan et al., 2017). In addition, irradiation also led to color and texture changes, lipid oxidation, unpleasant odor, and poor consumer acceptability (Duc et al., 2020). Likewise, treatments with chlorine and ozone have been reported due to the possibility of the development of hazardous and carcinogenic compounds such as trihalomethanes and chlorite in the produce and detrimental health effects upon exposure to high concentrations of ozone (Bai et al., 2019). Thus, the negative impacts of these physical and chemical methods have increased the need to search for and develop a novel biocontrol agent that is safe and eco-friendly without affecting the nutrients and quality of fresh produce.

The exploration of phages as biocontrol agents has increased as a natural and green technology for effectively controlling of foodborne pathogens in fresh produce (Oh and Park, 2017). Phage is a virus that can infect and kill target bacteria specifically (Zampara et al., 2017). A phage exhibits either lytic or lysogenic characteristics depending on the life cycle of a phage with host bacteria (Clokic et al., 2011; Gill and Hyman, 2010). The lytic phage rapidly replicates within the infected bacterial chromosome and eventually lyses the cells, resulting in the host's death. In contrast, the lysogenic phage can exist as a prophage within the host cell after its DNA integration into the bacterial chromosome and does not result in the lysis of the bacterial cells. Thus, lytic phage has been the preferred control of foodborne pathogens due to the ability to specifically infect and eventually lyse the host bacteria (Bai et al., 2019). Recently, several lytic phages have been investigated as biocontrol agents; however, most of them could infect only one bacterial genus (Moye et

al., 2018; Oh and Park, 2017; Wei et al., 2019). The narrow host range of phage becomes limited for the control of several foodborne pathogens simultaneously in fresh produce. Hence, development of phages with broad host range is more desirable and effective as biocontrol agents since they can simultaneously infect multiple bacterial genera (Ross et al., 2016). Although phages specific to either *Salmonella*, *Shigella*, or *E. coli* O157:H7 have been reported in both *in vitro* and *in vivo* experiments (Oh and Park, 2017; Sabouri et al., 2017; Wei et al., 2019), a lytic phage that could simultaneously infect multiple foodborne pathogens is rarely reported (Table S1). This study investigated that a lytic phage KFS-EC3 efficiently infects *E. coli* O157:H7, *Shigella sonnei*, and *Salmonella* Mission. Our findings lay the groundwork for providing further insights into phage studies for the simultaneous control of foodborne pathogens contaminating a food product.

The safety, lytic property, and novelty of the phage should be investigated by genomic analysis to be able to use phages as biocontrol agents (Newase et al., 2019; Sritha and Bhat, 2018). Whole genome sequencing provides information on the genetics of the isolated phages and highlights the safety of the phage usage by confirming the absence of genes encoding lysogenic, virulence, antibiotic, and allergenic factors in the genome. Furthermore, genomic characteristics can be compared to those of other reported phages to ascertain the novelty of the phage. Therefore, this study aimed to characterize the broad host range and bactericidal effect, and to genetically analyze the safety, novelty, and lytic property of the phage KFS-EC3 as a biocontrol agent.

## 2. Materials and methods

### 2.1. Bacterial strains

*Escherichia coli* O157:H7 ATCC 10536 was obtained

from the American Type of Culture Collection (ATCC) and used as an indicator strain for phage propagation. *E. coli* O157:H7 ATCC 10536 was cultivated in 25 mL of tryptic soy broth (TSB, Difco Laboratories Inc., Sparks, MD, USA) at 37°C for 16 h with gentle shaking. After washing three times with sterilized phosphate-buffered saline (PBS 1×, pH 7.4, Life Technologies Co., Carlsbad, CA, USA) by centrifuging at 7,000 ×g for 4 min at 4°C, the bacterial pellet was resuspended with PBS. The concentration of the bacterial suspension was adjusted to 8 log CFU/mL based on standard curves previously constructed by measuring optical density at 640 nm.

## 2.2. High-titer propagation and purification of phage

Phage KFS-EC3 was previously isolated from a slaughterhouse sewage sample (Daegu, Korea) (Kim et al., 2021). For its high-titer propagation, 1% (v/v) of *E. coli* O157:H7 ATCC 10536 suspension was mixed with 3 mL of TA broth (8 g/L nutrient broth, 5 g/L NaCl, 0.2 g/L MgSO<sub>4</sub>, 0.05 g/L MnSO<sub>4</sub>, and 0.15 g/L CaCl<sub>2</sub>), and the mixture was incubated at 37°C for 2 h. Subsequently, 1 mL of phage solution was added followed by incubation for 2 h at 37°C. After centrifuging the mixture at 6,000 ×g for 20 min at 4°C, the supernatant was filtered using a 0.20-μm cellulose acetate filter. This procedure was repeated with increasing amounts of TA broth. The final concentration of the propagated phage was measured using the plaque assay as follows: TA soft agar (0.4% agar) including 100 μL of the phage and 200 μL of the bacterial suspension (~8 log CFU/mL) was poured onto TSA plates and incubated at 37°C for 16 h. Afterward, phage purification was performed using the previously described method (Lee et al., 2020). The propagated phage was mixed with 10% (w/v) polyethylene glycol 6000 (Sigma-

Aldrich Co., St. Louis, MO, USA) and 10 mL of 1 M NaCl, and then precipitated by incubating at 4°C for 16 h. After centrifugation at 7,200 ×g for 20 min at 4°C, the obtained phage pellet was suspended in sodium chloride-magnesium sulfate (SM) buffer (50 mM/L Tris-HCl, 100 mM NaCl, and 10 mM MgSO<sub>4</sub>, pH 7.5). CsCl-gradient ultracentrifugation was then performed at 39,000 ×g for 2 h at 4°C. A bluish opalescent band was collected, dialyzed, and stored in a glass vial at 4°C for further characterization studies. The final concentration of the purified phage (hereafter referred to as KFS-EC3) was determined using the plaque assay expressed as a plaque-forming unit (PFU)/mL. After phage purification, plaque assay was performed against host strains of *E. coli* ATCC 10536, *Shigella sonnei* ATCC 9290, and *Salmonella* Mission to confirm the host specificity of the phage.

## 2.3. Morphological characteristics of KFS-EC3

The morphological characteristics of KFS-EC3 were previously observed (Kim et al., 2021), however, a contractile property of its tail was found in this study. For transmission electron microscopy (TEM, H-7100, Hitachi Ltd., Tokyo, Japan) analysis, a carbon-coated copper grid was dipped into 1 mL of KFS-EC3 for 1 min and then stained with 2% uranyl acetate (Sigma-Aldrich Co.). The phage on the grid was observed at an accelerating voltage of 100 kV with 40,000× magnification.

## 2.4. Polyvalent bactericidal activity of KFS-EC3

The phage was added to each bacterial suspension (~8 log CFU/mL) of *E. coli* O157:H7 ATCC 10536, *S. Mission*, *S. sonnei* ATCC 9290, and their cocktail (1:1:1, v/v) at MOIs of 0.001, 0.1, and 1.0 to evaluate the polyvalent bactericidal activity of KFS-EC3. SM buffer was used for comparison in the control. The

mixtures of the phage and bacterial suspension were incubated at 37°C for 4 h with gentle shaking. The viable bacterial concentration was determined by plate count method using xylose lysine deoxycholate (XLD, Difco Laboratories Inc.) and eosin methylene blue (EMB, Difco Laboratories Inc.) agar plates.

### 2.5. Whole genome sequencing and *in silico* analysis of KFS-EC3

The genomic DNA of KFS-EC3 was extracted using a Phage DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturer's instructions. The genome sequencing was performed using the Illumina Miseq platform with paired-end reads of 2×150 bp size. The sequenced reads were assembled to single contig using the SPAdes genome assembler (Illumina Inc., San Diego, CA, USA). The complete sequence of KFS-EC3 genome was available in the GenBank database under accession number MZ06535. The contig of KFS-SE3 genome was annotated using the Rapid Annotations Systems Technology (RAST) server (Aziz et al., 2008). The genome was analyzed for genes involved in host lysis and prophage to confirm the lytic cycle of KFS-EC3, using BLASTp, conserved domain database, and PHASTER (Arndt et al., 2016). In addition, potential virulence factors and antibiotic resistance genes were screened against the virulence factor database (Liu et al., 2022) and Comprehensive Antibiotic Resistance Database (<https://card.mcmaster.ca>, 5 November 2022), respectively.

### 2.6. Genomic comparative analysis of KFS-EC3

The phylogenetic analysis was performed to confirm the classification and novelty of KFS-EC3, followed by a comparison of genomic characteristics

with phylogenetically related phages. The amino acid sequences of similar myophages' tail proteins were obtained from the NCBI database and aligned using the ClustalW algorithm (Larkin et al., 2007). The UPGMA tree was constructed based on tail protein using Splits Tree4 (Huson and Bryant, 2010) with 1,000 bootstrap replicates. Additionally, the general genomic characteristics, identity, and ANI value of phylogenetically related phages were confirmed using the NCBI database, BLASTN analysis, and OrthoANI Tool v0.93 (Lee et al., 2016b), respectively.

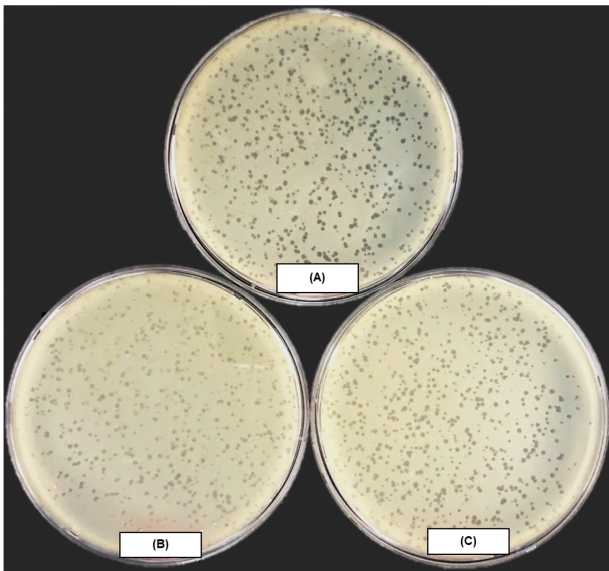
### 2.7. Statistical analysis

Experiments were conducted in triplicate and the data were expressed as mean±standard deviation (SD). Statistical analysis was performed using InStat V.9 (GraphPad, San Diego, CA, USA). Student's paired t-test and one-way analysis of variance (ANOVA) were used to compare in-between groups and among groups, respectively. Differences among means were analyzed using Duncan's Multiple Range Test (DMRT), and the significance level was defined at  $p < 0.05$ .

## 3. Results and discussion

### 3.1. Purification and morphological characteristics of KFS-EC3

KFS-EC3, a previously isolated phage, was propagated and purified at a concentration of  $(2.3 \pm 0.2) \times 10^{11}$  PFU/mL. As shown in Fig. 1, small and clear plaques of KFS-EC3 were observed on the lawn of *E. coli* ATCC 10536, *S. sonnei* ATCC 9290, and *S. Mission*, indicating its specificity with broad host range. Although there is no clear definition of between broad and narrow host range of phage, broad host range was often referred to phages



**Fig. 1.** Plaque formation of KFS-EC3 using plaque assay against (A) *E. coli* ATCC 10536, (B) *S. sonnei* ATCC 9290, and (C) *S. Mission*.

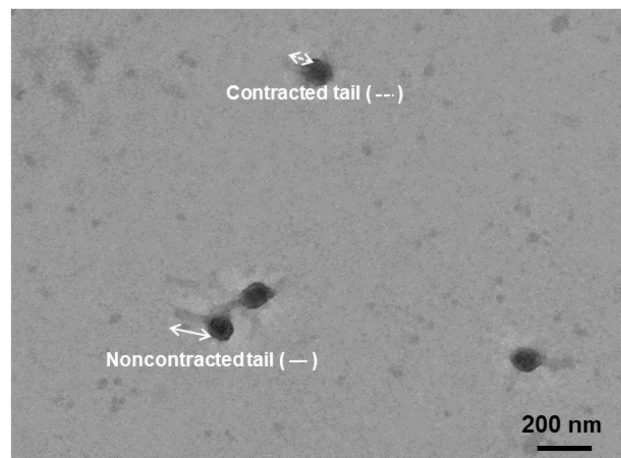
infesting more than two bacterial genera (Bielke et al., 2007; Kim et al., 2021). The broad host range of KFS-EC3 had benefits for practical use to control multiple foodborne pathogens in food, rather than reported phages with a narrow host range such as phage Phaxl (Shahrabak et al., 2013), phage phiLLS (Amarillas et al., 2017), and phage vB\_EcoS\_HSE2 (Peng and Yuan, 2018). Similar to this study, several studies have also showed lytic *E. coli* phages with broad host range (Table S1). Most of them can infect two bacterial genera such as phages PS5 (Duc et al., 2020), SFP10 (Park et al., 2012), vB\_EcoM\_swi3 (Sui et al., 2021), phiC119 (Amarillas et al., 2016), HY01 (Lee et al., 2016a) and SH6 (Hamdi et al., 2017), which means a narrower host range than the phage KFS-EC3. Some *E. coli* phages capable of infecting three genera have also been reported, such as phage EcS1 (Saad et al., 2019) and SH7 (Hamdi et al., 2017) specific to *E. coli*, *Salmonella* spp., and *Shigella* spp. Overall, a broad host range at the genus level of KFS-EC3 would be advantageous for the simultaneous

biocontrol of *E. coli* O157:H7, *Salmonella*, and *S. sonnei* in food.

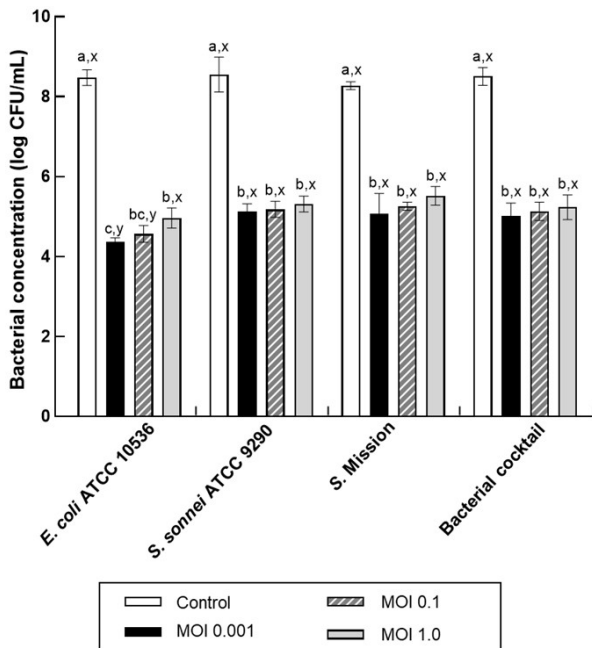
The morphological analysis by TEM showed that the KFS-EC3 phage had an icosahedral head with a length of  $97.1 \pm 5.2$  nm and a contractile tail with a length ranging from 67.2 to 135.5 nm (Fig. 2). KFS-EC3 was found to be myophage based on these characteristics. Compared with other reported myophages with broad host ranges (Table S1), the head and tail lengths of KFS-EC3 phage were longer than those of phage PS5 (Duc et al., 2020) and vB\_EcoM\_swi3 (Sui et al., 2021). Additionally, KFS-EC3 had a shorter head and longer tail length than that of phage HY01 (Lee et al., 2016a), EcS1 (Saad et al., 2019), and SH7 (Hamdi et al., 2017). Thus, our findings suggested that KFS-EC3 exhibits broad host range and unique morphological differences from other myophages reported infecting *E. coli*.

### 3.2. Polyvalent bactericidal activity of KFS-EC3

The polyvalent bactericidal activity of KFS-EC was evaluated and compared against each single host strain and their bacterial cocktail at various MOIs (Fig. 3). After phage treatment for 4 h at all MOIs,



**Fig. 2.** Morphology of KFS-EC3 observed using a transmission electron micrograph at a magnification of  $\times 40.0$  k. The bar indicates a magnification size of 200 nm.



**Fig. 3.** Polyvalent bactericidal activity of KFS-EC3 against *E. coli*, *S. sonnei*, *S. Mission*, and their bacterial cocktail at various MOIs. Values are mean $\pm$ SD (n=3). Different superscript letters within the same bacteria (a-c) and MOIs (x-y) indicate a significant difference (p<0.05).

significant reductions of each single host strains and their bacterial cocktail were observed compared to the control (p<0.05). Bactericidal activities of KFS-EC3 at the lowest MOI of 0.001 were more outstanding than at other MOIs of 0.1 and 1.0 with bacterial reductions of  $4.1\pm 0.1$ ,  $3.4\pm 0.2$ , and  $3.1\pm 0.2$  log CFU/mL against *E. coli* O157:H7, *S. sonnei*, and *S. Mission*, respectively. In addition, the bacterial reduction of *E. coli* O157:H7 was significantly greater than those of *S. sonnei*, and *S. Mission* at MOIs of 0.001 and 0.1. However, there are no significant differences in bacterial reduction of them at MOI of 1.0. Interestingly, KFS-EC3 also exhibited competitive reductions of bacterial cocktail by  $\sim 3.5\pm 0.3$  log CFU/mL reduction at all MOIs. Thus, KFS-EC3 showed excellent polyvalent bactericidal activity when exposed to both single-host bacteria and multi-host bacteria.

Similarly, a previous study (Lee et al., 2016a) reported that phage HY01 reduced each host bacteria of *E. coli* O157:H7 ATCC 43901 and *S. flexneri* 2a strain 2457T by  $\sim 4$  log CFU/mL at MOI of 10. However, since fresh produce can be contaminated with several pathogens (Alegbeleye et al., 2018), the polyvalent bactericidal activity of the phage against bacterial cocktail was critical for simultaneous biocontrol of the foodborne pathogens in food application. Park et al. (2012) reported that the polyvalent bactericidal activity of phage SFP10 against a bacterial cocktail of *S. Typhimurium* and *E. coli* O157:H7, which simultaneously reduced *S. Typhimurium* by 1.8 log CFU/mL and *E. coli* by 4.3 log CFU/mL at MOI of 100. Duc et al. (2020) also showed that phage PS5 effectively reduces *S. Enteritidis*, *S. Typhimurium*, *E. coli* O157:H7, and their bacterial cocktail. Phage PS5 treatment at MOI of 10,000 caused significant bacterial reduction by  $\sim 2$  log CFU/mL for *S. Enteritidis*, *S. Typhimurium*, and bacterial cocktail and by  $\sim 4$  log CFU/mL for *E. coli* O157:H7. Compared to those of other *E. coli* phages with broad host specificity, KFS-EC3 exhibited similar polyvalent bactericidal activity at lower MOI. A lower MOI of the phage has been reported to be more beneficial and economical for use in food application (Oh and Park, 2017). Our study revealed that KFS-EC showed excellent polyvalent bacterial inhibition against multiple foodborne pathogens and their cocktail even at a very low MOI of 0.001. These results indicate the potential of KFS-EC3 as a biocontrol agent with great effectiveness and efficiency for controlling foodborne pathogens.

### 3.3. Genomic analysis and in silico annotation of KFS-EC3

The genome of KFS-EC3 consisted of 166,440 bp

with a G+C content of 35.5%. Its genome comprised 122 hypothetical ORFs, 151 functional ORFs, and 8 tRNAs (Table 1). The annotated functional genes were categorized into five functional groups: including nucleotide metabolism, phage packaging, structure, host lysis, and additional functions (Table S2). Fifty-nine genes were confirmed to participate in nucleotide metabolism, such as RNA polymerase, DNA polymerase, DNA ligase, DNA helicase, ribonucleotide reductase, exo/endonuclease, transcriptional regulator, etc. They have a role in DNA replication, recombination, repair, and their regulation. Furthermore, sixteen genes involved in phage packaging were found, such as porter protein (ORF 115), phage prohead assembly and capsid scaffolding proteins (ORF 38, 42, 91, 107, 111-114, and 133), phage tail assembly and scaffolding proteins (ORF 95, 120, 136, and 138), and terminase (ORF 118 and 119). Especially, terminase is known to play an essential role in the initiation of phage packaging by DNA recognition, cleavage, and its translocation into phage prohead (Rao and Feiss, 2008; Shen et al., 2012).

The main components of the phage structure were also found at the posterior of the genome and

predicted to encode capsid (ORF 86, 109-110, and 139), neck (ORF 121 and 122), tail (ORF 69-73, 89-90, 116-117, and 123-124), and baseplate (ORF 96-102, 125-129, 131, and 132). Among these components of phage structure, tail fibers on the side of the phage tail can lead to phage attachment to the host bacterial membrane and its DNA injection, which are responsible for determining phage host specificity (Garcia-Doval and van Raaij, 2012). Interestingly, tail fiber proteins from ORF 70, 71, and 124 of KFS-EC3 were similar to those of *E. coli* phages PaulHMueller (QXV83448.1, 76.9%), ime09 (YP\_007004622.1, 100.0%), and T6 (YP\_010067303.1, 100.0%), respectively. Other fiber proteins from ORF 69, 72, and 73 were similar to those of *Shigella* phages A2 (UVD36934.1, 98.9%), KRT47 (QHB43271.1, 98.7%), and Esh26 (URY13523.1, 98.1%), whereas proteins from ORF 89 and 90 were similar to those of *Salmonella* phages Lv5cm (QVW09019.1, 98.7%) and SG1 (YP\_010075104.1, 98.0%). These results agreed with the conserved domain analysis of phage tail fibers (Table 2). Finally, our results suggested that these multiple tail fibers of KFS-EC3 may contribute to its broad host range against *E. coli*, *Shigella*, and *Salmonella*.

**Table 1.** Genomic characteristics of KFS-EC3 and other similar *E. coli* phages

Phage	Size (bp)	G+C (%)	tRNA	Identity (%)	ANI (%)	Accession
KFS-EC3	166,440	35.5	8	This study	-	MZ065353.1
Teqhal	168,070	35.1	11	96.28	95.32	MN895435.1
Kha5h	167,318	35.5	10	96.35	95.12	KT184312.1
Shf12	165,919	35.6	10	97.47	95.64	HM035025.1
vB_EcoM_KAW1E185	164,987	35.4	10	96.56	95.42	MK373781.1
Teqskov	165,017	35.4	6	96.69	95.73	MN895437.1
vB_EcoM_Ozark	167,600	39.5	10	96.49	95.47	MT682713.1
PhiZZ30	167,484	35.3	9	96.25	95.16	MT176426.1
vB_EcoM_Lutter	170,054	35.4	8	96.39	95.10	MT682714.1
T4	168,908	35.3	8	96.23	94.88	MT984581.1

**Table 2.** Annotated genes and their conserved domains associated with host lysis and tail fiber proteins of KFS-EC3

Function	Gene	Location (nt)	Strand	Conserved domain (accession)	E-value
Host lysis	Holin	68239–68895	–	Bacteriophage holin (pfam11031)	4.48e-111
	Endolysin	165129–165623	+	Endolysin and autolysin, T4-like lysozymes (cd00737)	2.12e-18
Phage tail fiber	Tail fiber	68927–69712	–	Tail fibre adhesion, Gp38 protein (pfam05268)	2.82e-68
	Tail fiber	69743–73639	–	Chaperone of endosialidase (pfam13884)	2.18e-11
	Tail fiber	73648–74313	–	Tail fiber (pfam03903)	5.93e-35
	Long tail fiber	74376–75491	–	Interleukin-like EMT inducer (pfam15711)	1.51e-05
	Long tail fiber	75500–79369	–	Long tail fiber, proximal subunit (PHA02584)	0e+00
	Tail fiber	96623–96859	+	ND <sup>1)</sup>	ND
	Tail fiber	96860–97168	+	Sm-like SH3 beta-barrel fold (pfam10902)	1.33e-17
	Long tail fiber	138435–140018	–	Receptor binding domain (pfam14928) and long tail fiber, proximal subunit (PHA02584)	3.49e-59 1.97e-20

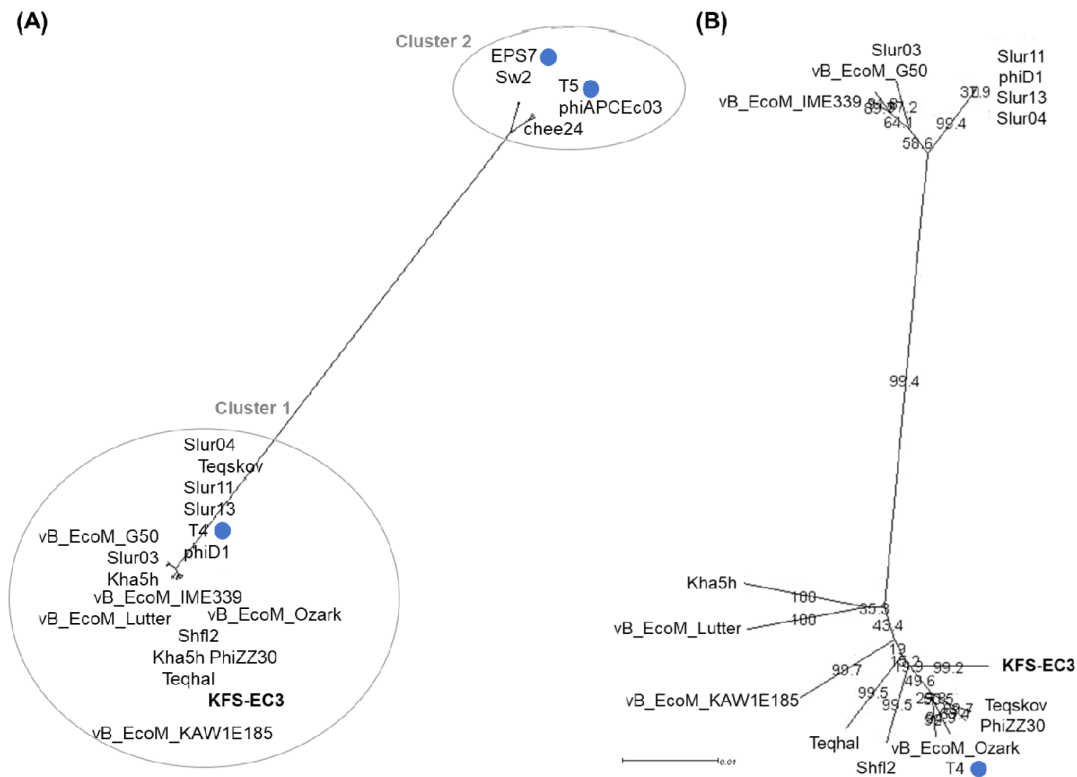
<sup>1)</sup>ND, not detected.

The lytic activity of KFS-EC3 was further evaluated by confirming host lysis proteins and prophage for use as a biocontrol agent. From the annotation and conserved domain analysis (Table 2 and S2), the genes involved in host lysis were identified as a holin (ORF 68) and endolysin (ORF 143). Phage endolysin can lyse the bacterial cell wall during the end of a lytic cycle for its release, which was supported by the holin to form holes in the bacterial membrane (Li et al., 2021). Most endolysins, which lyse gram-negative host bacteria, are structured to have a single domain of enzymatically active domain (EAD) (Abdelrahman et al., 2021). From the *in silico* analysis, the endolysin (ORF143) of KFS-EC3 belongs to the muramidase family comprised of one EAD, such as reported *E. coli* phages of T7 (Inouye et al., 1973), T4 (Matthews and Remington, 1974), and vB\_EcoM\_ECP26 (Park and Park, 2020). In addition, KFS-EC3 did not contain any genes associated with prophage, virulent factors, antibiotic resistance, and allergenicity. Overall, KFS-EC3 exhibited its potential as a lytic and safe biocontrol agent for food application.

### 3.4. Comparative genomic analysis of KFS-EC3

Phylogenetic analysis based on tail protein (Fig. 4(A)) revealed that similar *E. coli* myophages were divided into two groups. KFS-EC3 was clustered with 16 phages from the genus *Tequatrovirus* of the family Myoviridae (indicated as cluster 1), whereas cluster 2 contained phages of other genera *Tequintavirus* (phages T5, chee24, and phiAPCEc03) and *Epseptimavirus* (phages EPS7 and Sw2). In further comparison between phages of cluster 1 (Fig. 4(B)), KFS-EC3 was particularly related to 9 phages, including type phage T4 of *E. coli* phage. Then, genomic features, identity, and ANI value of KFS-EC3 were compared with these phylogenetically similar 9 phages (Table 1). These phages have a similar genome size of ~165–170 kbp, GC contents of ~35–40%, and tRNA of 6–10. A total of tRNAs in the KFS-EC3 genome was greater than phage Teqskov and similar to phages vB\_EcoM\_Lutter and T4. The presence of tRNA genes in the phage genome has been reported to enhance the protein translation efficiency in host bacteria and/or expand the host specificity (Grose et al., 2014;





**Fig. 4.** Phylogenetic tree based on the tail protein of (A) KFS-EC3 and other related phages and (B) phages grouped into cluster 1. ●, a type phage of its genera.

Morgado and Vicente, 2019). ANI analysis showed that KFS-EC also have a genetically close relationship with phages Teqskov (95.73%), Shfl2 (95.64%), and vB\_EcoM\_Ozark (95.47%). These ANI values were higher than the mechanistic demarcation criterion (70%) for phage genera (Turner et al., 2021). Overall, KFS-EC3 was classified as a new member of the *Tequatrovirus* genus of the Myoviridae family with genetically different characteristics from other reported phages.

#### 4. Conclusions

Phage-based biocontrol strategy is a promising approach for controlling foodborne pathogens in fresh produce. This study investigated the suitability of phage KFS-EC3 on the control of multiple

foodborne pathogens and its genomic characteristics for use as a biocontrol agent. KFS-EC3 showed efficient and effective bactericidal effect to reduce *E. coli* O157:H7, *S. sonnei*, and *S. Mission*, as well as their bacterial cocktail, indicating its potential for simultaneous biocontrol of these foodborne pathogens in fresh produce. The lytic property of the phage was also identified via genetic *in silico* analysis, which identified its host cell lysis genes and revealed the lack of genes related to prophage. Genomic *in silico* analysis revealed the safety of KFS-EC3 based on the absence of any genes associated with prophages, virulent factors, antibiotic resistance, and allergenicity. Furthermore, morphology and phylogenetic analyses revealed that KFS-EC3 is a new phage belonging to the Myoviridae family,

*Tequatrovirus* genus. Therefore, the lytic and safe phage KFS-EC3 is a new, promising candidate of biocontrol agent for use in fresh produce. Future studies will evaluate the control efficacy of the phage against these foodborne pathogens in fresh produce. It is also important to evaluate its stability on the surface of fresh produce, which would highlight its potential for application to the washing and sanitizing process of fresh produce.

### Supplementary materials

Supplementary materials are only available online from: <https://doi.org/10.11002/kjfp.2022.29.7.1022>.

### Acknowledgements

This research was supported by Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01572601) funded by Rural Development Administration, Korea.

### Conflict of interests

The authors declare no potential conflicts of interest.

### Author contributions

Conceptualization: Kim SH, Park MK. Methodology: Kim SH. Formal analysis: Kim SH, Park YR, Jung H. Validation: Kim SH, Park MK. Writing - original draft: Kim SH. Writing - review & editing: Park MK.

### Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

### ORCID

Su-Hyeon Kim (First author)

<https://orcid.org/0000-0001-9025-3543>

Ye-Rim Park

<https://orcid.org/0000-0001-9164-6912>

Hyeju Jung

<https://orcid.org/0000-0003-0859-5507>

Mi-Kyung Park (Corresponding author)

<https://orcid.org/0000-0003-1182-6046>

## References

- Abdelrahman F, Easwaran M, Daramola OI, Ragab S, Lynch S, Oduselu TJ, Khan FM, Ayobami A, Adnan F, Torrents E, Sanmukh S, El-Shibiny A. Phage-encoded endolysins. *Antibiotics*, 10, 124 (2021)
- Alegbeleye OO, Singleton I, Santana AS. Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. *Food Microbiol*, 73, 177-208 (2018)
- Amarillas L, Chaidez C, Gonzalez-Robles A, Lugo-Melchor Y, Leon-Felix J. Characterization of novel bacteriophage phic119 capable of lysing multidrug-resistant Shiga toxin-producing *Escherichia coli* O157: H7. *PeerJ*, 4, e2423 (2016)
- Amarillas L, Rubi-Rangel L, Chaidez C, González-Robles A, Lightbourn-Rojas L, León-Félix J. Isolation and characterization of phiLLS, a novel phage with potential biocontrol agent against multidrug-resistant *Escherichia coli*. *Front Microbiol*, 8, 1355 (2017)
- Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. PHASTER: A better, faster version of the PHAST phage search tool. *Nucleic Acids Res*, 44, W16-W21 (2016)
- Aziz RK, Bartels D, Best AA, Dejongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M. The RAST server: Rapid annotations using subsystems technology. *BMC Genom*, 9, 1-15 (2008)
- Bai J, Jeon B, Ryu S. Effective inhibition of *Salmonella* Typhimurium in fresh produce by a phage cocktail targeting multiple host receptors. *Food Microbiol*, 77, 52-60 (2019)
- Bielke L, Higgins S, Donoghue A, Donoghue D, Hargis B. *Salmonella* host range of bacteriophages

- that infect multiple genera. *Poult Sci*, 86, 2536-2540 (2007)
- Centers for Disease Control and Prevention. National outbreak reporting system (NORS). Available from: <https://wwwn.cdc.gov/norsdashboard/>. Accessed Feb. 3, 2022.
- Chaidez C, Lopez J, Vidales J, Castro-Del Campo N. Efficacy of chlorinated and ozonated water in reducing *Salmonella typhimurium* attached to tomato surfaces. *Int J Environ Health Res*, 17, 311-318 (2007)
- Clokier MR, Millard AD, Letarov AV, Heaphy S. Phages in nature. *Bacteriophage*, 1, 31-45 (2011)
- Duc HM, Son HM, Yi HPS, Sato J, Ngan PH, Masuda Y, Honjoh K-I, Miyamoto T. Isolation, characterization and application of a polyvalent phage capable of controlling *Salmonella* and *Escherichia coli* O157: H7 in different food matrices. *Food Res Int*, 131, 108977 (2020)
- Fan X, Huang R, Chen H. Application of ultraviolet C technology for surface decontamination of fresh produce. *Trends Food Sci Technol*, 70, 9-19 (2017)
- Garcia-Doval C, Van Raaij MJ. Structure of the receptor-binding carboxy-terminal domain of bacteriophage T7 tail fibers. *Proc Natl Acad Sci*, 109, 9390-9395 (2012)
- Gill JJ, Hyman P. Phage choice, isolation, and preparation for phage therapy. *Curr Pharm Biotechnol*, 11, 2-14 (2010)
- Große JH, Jensen GL, Burnett SH, Breakwell DP. Erratum to: Genomic comparison of 93 *Bacillus* phages reveals 12 clusters, 14 singletons and remarkable diversity. *Bmc Genom*, 15, 1-20 (2014)
- Hamdi S, Rousseau GM, Labrie SJ, Tremblay DM, Kourda RS, Ben Slama K, Moineau S. Characterization of two polyvalent phages infecting Enterobacteriaceae. *Sci Rep*, 7, 1-12 (2017)
- Huson DH, Bryant D. User manual for splitstree4 V4. 11.3. (2010)
- Inouye M, Arnheim N, Sternglanz R. Bacteriophage t7 lysozyme is an n-acetylmuramyl-l-alanine amidase. *J Biol Chem*, 248, 7247-7252 (1973)
- Kim SH, Adeyemi DE, Park MK. Characterization of a new and efficient polyvalent phage infecting *E. coli* O157: H7, *Salmonella* spp., and *Shigella sonnei*. *Microorganisms*, 9, 2105 (2021)
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. Clustal W and clustal X version 2.0. *Bioinformatics*, 23, 2947-2948 (2007)
- Lee C, Choi IY, Park DH, Park MK. Isolation and characterization of a novel *Escherichia coli* O157:H7-specific phage as a biocontrol agent. *J Environ Health Sci Eng*, 18, 189-199 (2020)
- Lee H, Ku HJ, Lee DH, Kim YT, Shin H, Ryu S, Lee JH. Characterization and genomic study of the novel bacteriophage hy01 infecting both *Escherichia coli* O157:H7 and *Shigella flexneri*: Potential as a biocontrol agent in food. *PLoS One*, 11, e0168985 (2016)
- Lee I, Kim YO, Park SC, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol*, 66, 1100-1103 (2016)
- Li X, Zhang C, Wei F, Yu F, Zhao Z. Bactericidal activity of a holin-endolysin system derived from *Vibrio alginolyticus* phage HH109. *Microb Pathog*, 159, 105135 (2021)
- Liu B, Zheng D, Zhou S, Chen L, Yang J. VFDB 2022: A general classification scheme for bacterial virulence factors. *Nucleic Acids Res*, 50, D912-D917 (2022)
- Matthews B, Remington S. The three dimensional structure of the lysozyme from bacteriophage T4. *Proc Natl Acad Sci*, 71, 4178-4182 (1974)
- Molins R, Motarjemi Y, Kaferstein F. Irradiation: A critical control point in ensuring the microbiological safety of raw foods. *Food control*, 12, 347-356 (2001)

- Morgado S, Vicente AC. Global *in-silico* scenario of tRNA genes and their organization in virus genomes. *Viruses*, 11, 180 (2019)
- Moye ZD, Woolston J, Sulakvelidze A. Bacteriophage applications for food production and processing. *Viruses*, 10, 205 (2018)
- Newase S, Kapadnis BP, Shashidhar R. Isolation and genome sequence characterization of bacteriophage vB\_SalM\_PM10, a *Cba120virus*, concurrently infecting *Salmonella enterica* serovars Typhimurium, Typhi, and Enteritidis. *Curr Microbiol*, 76, 86-94 (2019)
- Oh JH, Park MK. Recent trends in *Salmonella* outbreaks and emerging technology for biocontrol of *Salmonella* using phages in foods: A review. *J Microbiol Biotechnol*, 27, 2075-2088 (2017)
- Park DW, Park JH. Characterization of endolysin LysECP26 derived from rv5-like phage vB\_EcoM-ECP26 for inactivation of *Escherichia coli* O157: H7. *J Microbiol Biotechnol*, 30, 1552-1558 (2020)
- Park M, Lee JH, Shin H, Kim M, Choi J, Kang DH, Heu S, Ryu S. Characterization and comparative genomic analysis of a novel bacteriophage, SFP10, simultaneously inhibiting both *Salmonella enterica* and *Escherichia coli* O157:H7. *Appl Environ Microbiol*, 78, 58-69 (2012)
- Peng Q, Yuan Y. Characterization of a newly isolated phage infecting pathogenic *Escherichia coli* and analysis of its mosaic structural genes. *Sci Rep*, 8, 1-10 (2018)
- Petri E, Rodríguez M, Garcia S. Evaluation of combined disinfection methods for reducing *Escherichia coli* O157:H7 population on fresh-cut vegetables. *Int J Environ Res Public Health*, 12, 8678-8690 (2015)
- Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerging Infect Dis*, 11, 603-609 (2005)
- Rao VB, Feiss M. The bacteriophage DNA packaging motor. *Annu Rev Genet*, 42, 647-681 (2008)
- Ruengvisesh S, Kerth CR, Taylor TM. Inhibition of *Escherichia coli* O157:H7 and *Salmonella enterica* isolates on spinach leaf surfaces using eugenol-loaded surfactant micelles. *Foods*, 8, 575 (2019)
- Saad AM, Soliman AM, Kawasaki T, Fujie M, Nariya H, Shimamoto T, Yamada T. Systemic method to isolate large bacteriophages for use in biocontrol of a wide-range of pathogenic bacteria. *J Biosci Bioeng*, 127, 73-78 (2019)
- Sabouri S, Sepehrizadeh Z, Amirpour-Rostami S, Skurnik M. A minireview on the *in vitro* and *in vivo* experiments with anti-*Escherichia coli* O157:H7 phages as potential biocontrol and phage therapy agents. *Int J Food Microbiol*, 243, 52-57 (2017)
- Schweiggert U, Carle R, Schieber A. Conventional and alternative processes for spice production: A review. *Trends Food Sci Technol*, 18, 260-268 (2007)
- Shahin K, Bouzari M. Bacteriophage application for biocontrolling *Shigella flexneri* in contaminated foods. *J Food Sci Technol*, 55, 550-559 (2018)
- Shahrbabak SS, Khodabandehlou Z, Shahverdi AR, Skurnik M, Ackermann H-W, Varjosalo M, Yazdi MT, Sepehrizadeh Z. Isolation, characterization and complete genome sequence of PhaxI: A phage of *Escherichia coli* O157: H7. *Microbiology*, 159, 1629-1638 (2013)
- Shen X, Li M, Zeng Y, Hu X, Tan Y, Rao X, Jin X, Li S, Zhu J, Zhang K, Hu F. Functional identification of the DNA packaging terminase from *Pseudomonas aeruginosa* phage PaP3. *Arch Virol*, 157, 2133-2141 (2012)
- Sritha K, Bhat SG. Genomics of *Salmonella* phage  $\phi$ Stp1: Candidate bacteriophage for biocontrol. *Virus Genes*, 54, 311-318 (2018)
- Sui B, Han L, Ren H, Liu W, Zhang C. A novel polyvalent bacteriophage vB\_EcoM\_swi3 infects pathogenic *Escherichia coli* and *Salmonella* Enteritidis. *Front Microbiol*, 12, 649673 (2021)
- Turner D, Kropinski AM, Adriaenssens EM. A

- roadmap for genome-based phage taxonomy. *Viruses*, 13, 506 (2021)
- Wei S, Chelliah R, Rubab M, Oh DH, Uddin MJ, Ahn J. Bacteriophages as potential tools for detection and control of *Salmonella* spp. in food systems. *Microorganisms*, 7, 570 (2019)
- Zampara A, Sorensen MCH, Elsser-Gravesen A, Brondsted L. Significance of phage-host interactions for biocontrol of *Campylobacter jejuni* in food. *Food Control*, 73, 1169-1175 (2017)