Journal of Microbiology (2019) Vol. 57, No. 2, pp. 170–179
DOI 10.1007/s12275-019-8610-0
eISSN 1976-3794
pISSN 1225-8873

Lytic KFS-SE2 phage as a novel bio-receptor for *Salmonella* Enteritidis detection[§]

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(Received Nov 8, 2018 / Revised Nov 26, 2018 / Accepted Dec 3, 2018)

Since *Salmonella* Enteritidis is one of the major foodborne pathogens, on-site applicable rapid detection methods have been required for its control. The purpose of this study was to isolate and purify *S.* Enteritidis-specific phage (KFS-SE2 phage) from an eel farm and to investigate its feasibility as a novel, efficient, and reliable bio-receptor for its employment. KFS-SE2 phage was successfully isolated at a high concentration of (2.31 ± 0.43) × 10^{11} PFU/ml, and consisted of an icosahedral head of 65.44 ± 10.08 nm with a non-contractile tail of 135.21 ± 12.41 nm. The morphological and phylogenetic analysis confirmed that it belongs to the *Pis4avirus* genus in the family of *Siphoviridae*. KFS-SE2 genome consisted of 48,608 bp with 45.7% of GC content. Genome analysis represented KFS-SE2 to have distinctive characteristics as a novel phage. Comparative analysis of KFS-SE2 phage with closely related strains confirmed its novelty by the presence of unique proteins. KFS-SE2 phage exhibited excellent specificity to *S.* Enteritidis and was stable under the temperature range of 4 to 50°C and pH of 3 to 11 ($P < 0.05$). The latent time was determined to be 20 min. Overall, a new lytic KFS-SE2 phage was successfully isolated from the environment at a high concentration and the excellent feasibility of KFS-SE2 phage was demonstrated as a new bio-receptor for *S.* Enteritidis detection.

***Keywords*:** bio-receptor, phage, *Salmonella* Enteritidis, lytic, biosensor

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[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

Introduction

Salmonella spp. is recognized as one of the major foodborne pathogens (Batz *et al.*, 2012). The annual cost associated with salmonellosis in 2010 was estimated at $2.71 billion for 1.4 million reported cases (USDA, 2013). More than 2,600 *Salmonella* serovars have been identified, of which, five main serotypes such as *Salmonella* Typhimurium, Enteritidis, Newport, Javiana, and Heidelberg, account for 56% of all *Salmonella* infections in human (Hungaro *et al.*, 2013). The most common symptoms of salmonellosis are diarrhea, fever, abdominal cramps, nausea, occasional vomiting, and headache (Oh and Park, 2017). Although *Salmonella* outbreaks have mainly been associated with eggs, poultry, pork, beef, and dairy products, its recent outbreaks have been reported to be caused by fresh produces (almost 4.3 folds increase from 2008 to 2017) (Doyle and Buchanan, 2012; Putturu *et al.*, 2015). Since fresh produces can be contaminated at any point during processing and undergo simple cooking and processing procedures, an on-site applicable rapid detection method of *Salmonella* is keenly needed for the prevention of *Salmonella* outbreaks (Li *et al.*, 2010; Park *et al.*, 2013a).

Although a conventional detection method has been considered as the "gold-standard method", its laborious and time consuming procedures provide some limitations for its use as an on-site applicable method (Park *et al.*, 2011; Sharma and Mutharasan, 2013). It is noteworthy that numerous commercial rapid detection methods using immunology-based products (*Salmonella* TEK, TECRA, Equate, BacTrace, VIDAS, and OPUS, etc.) and nucleic acid-based products (BAX, VIDAS, Lateral Flow System, etc.) have been popularly used. However, these methods also have some disadvantages such as complicated sample preparation and procedures and costly reagents and equipment, and so deemed unsuitable for on-site and in-field application (Park *et al.*, 2013b; Byeon *et al.*, 2015).

Biosensor methods consist of two major components: a bio-receptor which identifies and interacts with a target pathogen specifically and a transducer that converts the interaction of bio-receptor with target into a measurable signal (Singh *et al.*, 2013; Byeon *et al.*, 2015; Park and Chin, 2016). These biosensor methods are very economical and convenient and have recently been developed as a novel on-site applicable method (Singh *et al.*, 2013). To develop a biosensor method, an appropriate and optimum bio-receptor is key to play a pivotal role in respect of the novelty, sensitivity, specificity, and reliability of a new biosensor. There are several candidates employed in biosensor methods such as antibodies, bacteriophage, nucleic acid, and aptamer (Singh *et al.*, 2013). Among them, antibodies are highly available commercially, but these antibodies are unstable under adverse environmen-

tal conditions (temperature and pH) so its application as an on-site applicable bio-receptor in biosensor is limited (Olsen *et al.*, 2006; Velusamy *et al.*, 2010; Sorokulova *et al.*, 2014).

Bacteriophage (phages) have been recommended due to their excellent specificity, low inherent toxicity, adaptability to their host system, robustness against harsh environments, and relatively cheap and easy production procedures (Sillankorva *et al.*, 2012; Singh *et al.*, 2013; Byeon *et al.*, 2015). Relatively few phages have been employed in the amperometric biosensor, fluorescent biosensor, bioluminescent biosensor, impedimetric biosensor, quartz crystal microbalance (QCM), as well as surface plasmon resonance (SPR) for the detection of foodborne pathogens (Singh *et al.*, 2012). These employed phages are either an environmentally isolated phage with/without genetic modification or a filamentous phage obtained from phage typing technology (Sun *et al.*, 2001; Gervais *et al.*, 2007; Li *et al.*, 2010; Park *et al.*, 2013b). However, these genetic modifications and phage typing technologies require time- and labor-consuming procedures. In addition, when a filamentous phage (long thread-like structure) was employed as a bio-receptor, the filamentous phage showed an aggregation problem due to lots of binding sites surrounding both sides of the phage (Hosseinidoust *et al.*, 2014; Hiremath *et al.*, 2015). Several trials have been performed to isolate a new wild-type phage for direct employment to various biosensor methods in order to overcome these limitations (Singh *et al.*, 2009; Arya *et al.*, 2011). Hence, a new *S.* Enteritidis-specific phage (hereafter referred to as KFS-SE2 phage) was explored and investigated for its feasibility as a novel bio-receptor for its employment as an on-site applicable biosensor method.

Materials and Methods

Bacterial strains and growth conditions

Each bacterial strain (Table 1) was grown in 25 ml of tryptic soy broth (TSB, Difco Laboratories Inc.) at 37°C for 16 h. The optical density of each overnight culture was measured at 640 nm to adjust its final concentration to 10^8 CFU/ml for the specificity test. For plaque assay, the overnight culture of *Salmonella* Enteritidis ATCC 13076 was washed three times by centrifugation at 4,000 × *g* for 4 min at 4°C. Each collected bacterial cell was then suspended in sterilized phosphate-buffered saline (PBS, pH 7.4, Life technologies Co.) and the final bacterial concentration was adjusted to 10^8 CFU/ml by using a pre-constructed standard curve at 640 nm.

Isolation, propagation, and purification of KFS-SE2 phage

The overnight culture of *S.* Enteritidis ATCC 13076, as an indicator strain, was mixed with 250 ml of TSB containing 25 ml of water sample obtained from an eel farm (Jinju, Gyeongsangnam-do, Korea) and incubated at 37°C for 16 h. The mixture was centrifuged at 4,000 × *g* for 10 min at 4°C and the supernatant was filtered using a cellulose acetate filter (0.20-μm pore size, Advantec Toyo Ltd.). To identify phages from the water sample, 10 μl of the filtrate was dropped on the surface of pre-solidified TA soft agar (4 g agar, 8 g nutrient broth, 5 g NaCl, 0.2 g $MgSO_4$, 0.05 g $MnSO_4$, and 0.15 g $CaCl_2$/L) containing 200 μl overnight culture of indicator strain for incubation (dot assay). Once the clear zone was formed, the single phage was isolated from the filtrate by using plaque assay. One hundred microliters of the filtrate with serial dilutions was mixed with 200 μl of centrifuged overnight culture of indicator strain and 4 ml of TA soft agar prior to pouring onto tryptic soy agar (TSA, Difco Laboratories Inc.) plate for incubation at 37°C (plaque assay). A single plaque was then eluted with sodium chloride-magnesium sulphate (SM) buffer (50 mM Tris-HCl, 100 mM NaCl, 10 mM $MgSO_4$, pH 7.5) with agitation (Gwak *et al.*, 2018). For the propagation of the isolated single phage, 1% overnight cul-

Table 1. Specificity of KFS-SE2

Bacterial strains	Plaque formation*	Source
Salmonella spp.		
Salmonella Enteritidis ATCC 13076	+	ATCC
S. Enteritidis NCCP 14771	+	NCCP
S. Enteritidis NCCP 14545	+	NCCP
S. Enteritidis NCCP 14546	+	NCCP
S. Dublin	-	DPFS
S. Hartford	-	DPFS
S. Heidelberg	-	DPFS
S. Mission	-	DPFS
S. Montevideo	-	DPFS
S. Newport	-	DPFS
S. Panama	-	DPFS
S. Salamae	-	DPFS
S. Senftenberg	-	DPFS
S. Typhimurium	-	DPFS
Non-*Salmonella* strains		
Aeromonas hydrophila ATCC 7966	-	ATCC
Bacillus cereus ATCC 13061	-	ATCC
B. cereus ATCC 21768	-	ATCC
B. cereus ATCC14579	-	ATCC
B. pumilus SR067	-	DPFS
B. safensis SR138	-	DPFS
B. subtilis ATCC 6633	-	ATCC
B. toyonensis SR070	-	DPFS
Escherichia coli O157:H7 ATCC 43895	-	ATCC
E. coli O157:H7 rif R	-	DPFS
E. coli O157:H7 204p	-	DPFS
E. coli ATCC BAA-2192	-	ATCC
E. coli ER 2738	-	DPFS
Klebsiella pneumoniae ATCC 13883	-	ATCC
Listeria monocytogenes ATCC 19111	-	ATCC
L. innocua ATCC 33090	-	ATCC
Pseudomonas aeruginosa ATCC 9027	-	ATCC
Staphylococcus aureus ATCC 25923	-	ATCC
Shigella boydii NCCP 11190	-	NCCP
S. flexneri 2457	-	DPFS
S. sonnei ATCC 9290	-	ATCC
Vibrio parahaemolyticus ATCC 17802	-	ATCC
Yersinia enterocolitica ATCC 23715	-	ATCC

* +, clear plaque; -, no plaque
ATCC, American Type of Culture Collection; DPFS, Department of Plant and Food Sciences, Sangmyung University, Cheonan, Republic of Korea; NCCP, National Culture Collection for Pathogens

ture of indicator strain was mixed with 3 ml of TA broth and incubated at 37°C for 2.5 h with agitation. Then the isolated single phage was added and incubated at 37°C for 2 h, followed by centrifugation at 4,000 × *g* for 10 min and filtration. The above procedures were performed several times by increasing the amount of TA broth for increasing the concentration of the isolated single phage. The final filtrate was mixed with 10% (w/v) of polyethylene glycol (PEG) 6000 (Sigma-Aldrich Co.) and 10 ml of 1 M NaCl at 4°C for precipitation of the phage. Then, centrifugation was performed at 7,200 × *g* (20 min) and the pellet was suspended in SM buffer followed by CsCl gradient ultracentrifugation at 39,000 × *g* at 4°C for 2 h. Finally, the layer of bluish opalescent band was dialyzed and stored in SM buffer at 4°C and the final phage concentration was measured by plaque assay.

Morphological analysis of KFS-SE2 phage

Purified KFS-SE2 phage was dropped on a carbon-coated copper grid for adsorption and then stained with 2% phosphotungstic acid (Sigma-Aldrich Co.). The stained KFS-SE2 phage was observed using a transmission electron microscope (TEM, H-7100, Hitachi Ltd.) at 100 kV with 70,000 to 200,000 × magnifications.

Genome sequencing and annotation of KFS-SE2 phage

Genomic DNA of KFS-SE2 phage was extracted using the Phage DNA Isolation Kit (Norgen Biotek Co.) according to the manufacturer's instructions. Genome sequencing was performed by LabGenomics using the Miseq platform (Illumina, Inc.). Of the total 2 × 1,636,751 reads, raw reads were trimmed using BBduk trimmer (version 1.0) to eliminate low quality read and adaptor sequences (Bushnell, 2014). Trimmed reads were assembled into contigs using Geneious (version 11.1.5.; Biomatters Ltd.). Briefly, 2,272,824 of 3,273,502 reads were assembled and produced 12,476 contigs, which were used to reconstruct a 48,608 bp consensus sequence. The genome sequence was annotated by Rapid Annotations using Subsystem Technology (RAST) (Aziz *et al.*, 2008) and confirmed with PHASTER (Arndt *et al.*, 2016). The packaging mode and the nature of the KFS-SE2 genome termini were determined using PhageTerm software (version 1.0.12) with default options (Garneau *et al.*, 2017). All identified open reading frames (ORFs) were compared against the Comprehensive Antibiotic Resistance Database (CARD) (McArthur *et al.*, 2013), the ResFinder 2.1 (Zankari *et al.*, 2012), and the virulence factor database (Chen *et al.*, 2016). The predicted KFS-SE2 protein sequences were compared against the allergen database (http://www.allergenonline.com) from Food Allergy Research. A genome map was generated and annotated according to the RAST subsystem. The complete genome sequence of KFS-SE2 was made available in the GenBank database under nucleotide sequence accession number MK-112901.

Phylogenetic analysis of KFS-SE2 phage

The genome sequence of KFS-SE2 was initially compared with those available in the GenBank database using the PubMed NCBI BLAST program. Fourteen phage genome sequences from the GenBank database were included for phylogenetic analysis. These phages were selected as the closely related genome sequences with KFS-SE2 (10 genome sequences) and the representatives of genera (4 genome sequences) within family *Siphoviridae* (Lefkowitz *et al.*, 2017). The KFS-SE2 genome sequence with 14 phage sequences was subjected to genome-based phylogenetic analysis using the Virus Classification and Tree building Online Resource (VICTOR) (Meier-Kolthoff and Göker, 2017). All pairwise comparisons of the nucleotide sequences were performed using Genome-BLAST Distance Phylogeny (GBDP) method with settings for prokaryotic viruses (Meier-Kolthoff *et al.*, 2014). The resulting intergenomic distances were used to infer a balanced minimum evolution tree with branch support using FASTME including SPR post-processing (Lefort *et al.*, 2015). Branch support was inferred from 100 pseudo-bootstrap replicates each. Trees were rooted at midpoint (Farris, 1974) and visualized with FigTree (Rambaut, 2017). Taxon boundaries at the genus level were determined with the OPTSIL program (Göker *et al.*, 2009), the recommended clustering thresholds (Meier-Kolthoff and Göker, 2017), and an F value (fraction of links required for cluster fusion) of 0.5 (Meier-Kolthoff *et al.*, 2014). KFS-SE2 was classified according to International Committee on Taxonomy of Viruses (ICTV) taxonomic assignments (Lefkowitz *et al.*, 2017).

Comparative analysis of annotated KFS-SE2 proteins

Nineteen annotated proteins of KFS-SE2 were analyzed using the NCBI BLASTP program to compare with relative strains based on phylogenetic analysis. Heatmap was generated to visualize coverage and identity among relative phage strains using MORPHEUS (https://software.broadinstitute.org/morpheus).

Specificity of KFS-SE2 phage

Two hundred microliters of each overnight culture of bacterial strain (Table 1) was mixed with 4 ml of TA soft agar and poured on the pre-solidified TSA plate. Ten microliters of the 10-fold diluted KFS-SE2 phage was dotted on the prepared plate above. After incubation at 37°C for 16 h, the formation of plaque against each strain was observed and compared.

Stability study of KFS-SE2 phage

To investigate the stability of KFS-SE2 phage against environmental factors, 100 µl of KFS-SE2 phage (10^8 PFU/ml) was mixed with 900 µl of TSB. After being placed in various temperatures (-20, 4, 22, 37, 50, 60, and 70°C) for 1 h or pHs (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 12) at 22°C for 1 h, the plaque assay was carried out to compare phage stability.

Measurement of latent time of KFS-SE2 phage

The overnight culture of indicator strain (10^8 CFU/ml) and KFS-SE2 phage (10^5 PFU/ml) were mixed and incubated for 10 min at 22°C in order to allow the phage adsorption into *S.* Enteritidis. The mixture was centrifuged at 11,400 × *g* for 10 min at 4°C and the pellet (infected bacterial cells) was resuspended with 10 ml of TSB for incubation at 37°C for 1 h.

At every five-minute interval, two 0.5 ml suspensions were taken from the incubated suspensions and 1% chloroform (v/v) was added into only one of the two suspensions. Plaque assay was then performed to determine the latent time.

Statistical analysis

The experiments were replicated three times and the experimental results were expressed as mean ± standard deviations (SD). The one-way analysis of variance (ANOVA) among more than two groups was run to compare the means using GraphPad Prism 5 and InStatV.3 programs (GraphPad Software Inc.). The significance level was determined at $P < 0.05$.

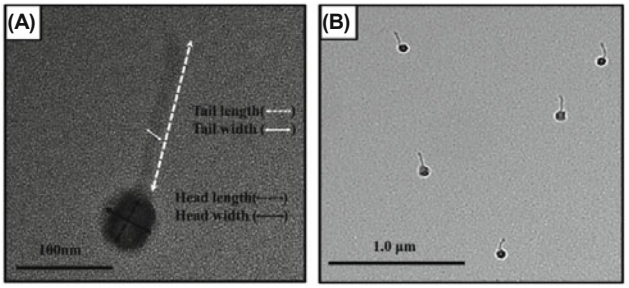

Fig. 1. TEM images of KFS-SE2 phage at a magnification of (A) × 70.0 k and (B) × 20.0 k.

Results

Isolation and purification of KFS-SE2 phage and its morphological characteristics

Six phages infecting *S.* Enteritidis were isolated from environmental samples collected from an eel farm and six slaughterhouses (Daegu, Gunwi, Gumi, Andong, Gyeongsan, and Yeongcheon, Korea). Based on our preliminary study, an eel farm isolated phage was selected for further study due to the clearest and largest plaque formation. KFS-SE2 phage was isolated and purified from an eel farm at a high concentration of $(2.31 \pm 0.43) \times 10^{11}$ PFU/ml. TEM analysis (Fig. 1) revealed that KFS-SE2 phage consisted of an icosahedral head with length of 65.44 ± 10.08 nm and width of 66.83 ± 8.51 nm, and a tail with length of 135.21 ± 12.41 nm and width of 14.76 ± 2.03 nm. From the several TEM images, the tail length of KFS-SE2 phage was constant, indicating a non-contractible tail. Thus, KFS-SE2 was assumed to be classified into one of *Siphoviridae* family based on the morphological comparisons with other previous studies (Choi *et al.*, 2017).

Genome analysis of KFS-SE2 phage

The genome of KFS-SE2 phage was sequenced with 14,027 × coverage. The assembled KFS-SE2 genome consists of 48,608 bp and its GC content is 45.8% (Fig. 2). KFS-SE2 phage has terminally redundant ends of 1,398 bp and is predicted to be permuted by PhageTerm (Garneau *et al.*, 2017). In total, 90 open reading frames (ORFs) and two tRNA gene were identified in KFS-SE2 genome. Of these ORFs, only 19 ORFs were annotated as phage proteins and 71 ORFs remained

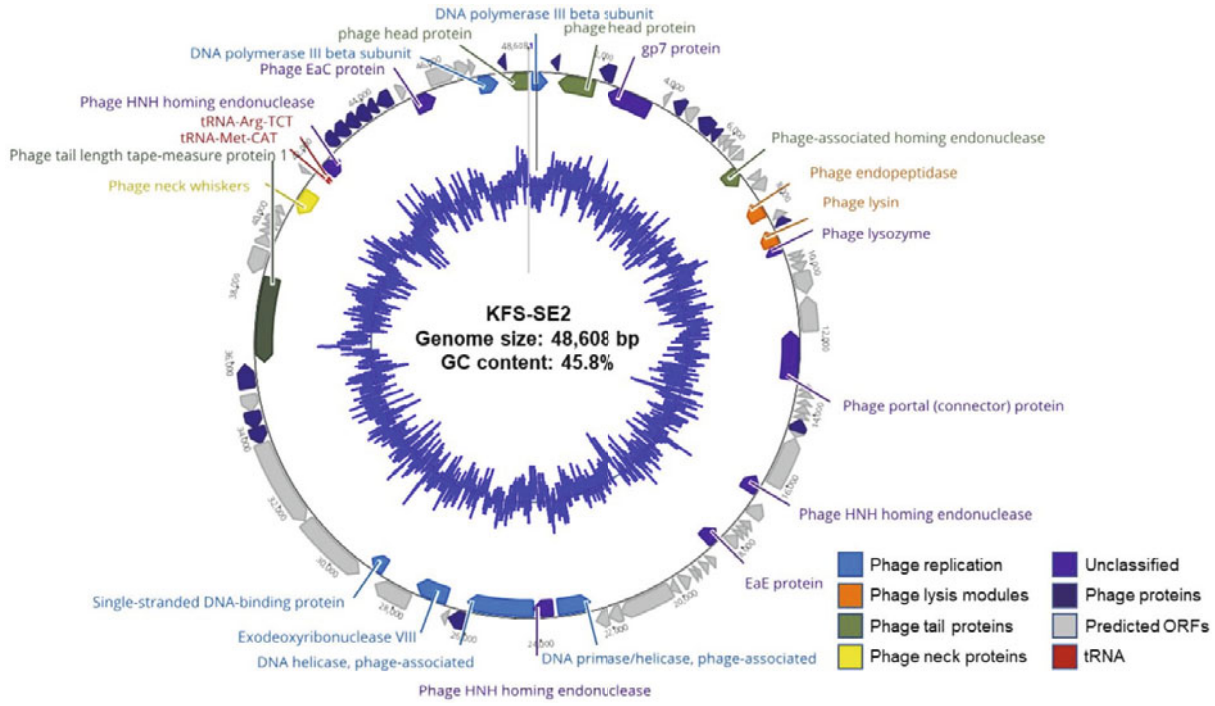

Fig. 2. Genome organization of KFS-SE2. Linear genome of KFS-SE2 was circularized to describe genomic feature. Predicted ORFs are indicated as arrows. The head of arrows designate direction of transcription. The colors were assigned according to subsystem classification (or functional categories) of the ORFs in the RAST pipeline. Grey arrows indicate ORFs encoding putative or hypothetical proteins. The blue circle designates GC content. More detailed annotation information is listed in Supplementary data Table S1.

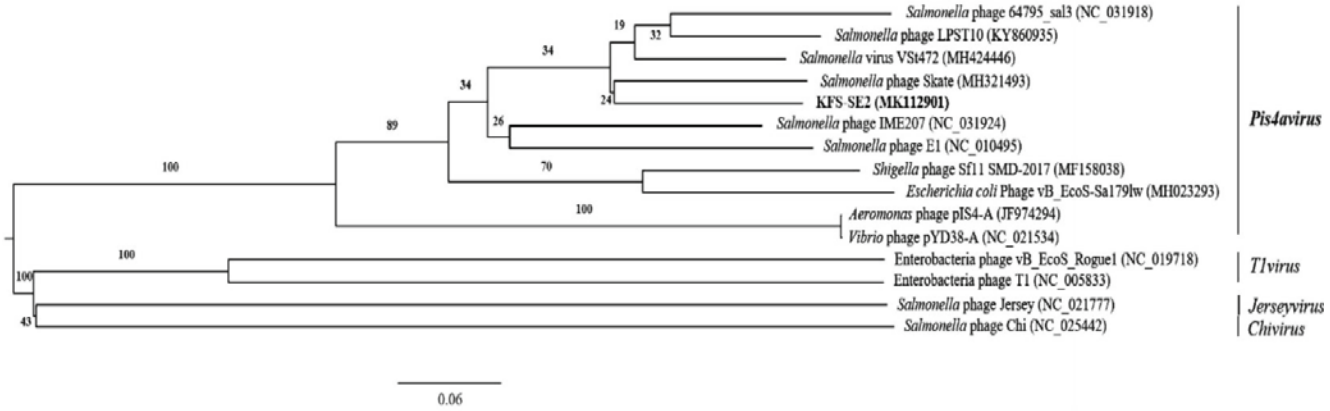

Fig. 3. Phylogenetic tree of KFS-SE2 with representative phage strains. KFS-SE2 is shown in the phylogenomic GBDP trees inferred using the formulas D0 and yielding an average support of 55%. The numbers above the branches are GBDP pseudo-bootstrap support values from 100 replications. The branch lengths of the resulting VICTOR trees are scaled in terms of the respective distance formula used. The numbers in parentheses are GenBank sequence accession numbers. Genus classification by ICTV taxonomic assignments (Lefkowitz *et al*., 2017) is listed on the right.

putative proteins and hypothetical proteins (Fig. 2 and Supplementary data Table S1). Functional genes involved in phage binding with *S*. Enteritidis were not found. Two tRNA genes (tRNA-Met-CAT and tRNA-Arg-TCT) were found in the KFS-SE2 genome (Fig. 2 and Supplementary data Table S1). More importantly, no genes were found for lysogenic activity, virulence, antibiotic resistance, and potential allergens in the KFS-SE2 genome.

Phylogenetic analysis of KFS-SE2 phage

The genome sequence of KFS-SE2 showed that it has low coverage (< 74%) and identity (< 94%) with available complete genomes of phages within *Siphoviridae* in a nucleotide BLAST comparison (Supplementary data Table S2). However, a phylogenetic analysis with genome sequences of representative phages within *Siphoviridae* (Fig. 3) revealed that KFS-SE2 is closely related to strains within the genus *Pis4avirus* (Göker *et al.*, 2009), as proposed by ICTV (Lefkowitz *et al.*, 2017).

Comparative analysis of annotated KFS-SE2 proteins

Annotated proteins were subjected to NCBI BLASTP search. We compared KFS-SE2 ORFs with closely related strains (*Salmonella* phage Skate, *Salmonella* phage 62795_sal3, *Salmonella* phage LPST10, *Salmonella* virus VSt472, and *Escherichia* phage vB_EcoS Sal179lw) based on phylogenetic analysis (Fig. 3) and represented query coverage and identified on heatmaps for comparison. Heatmaps of coverage and iden-

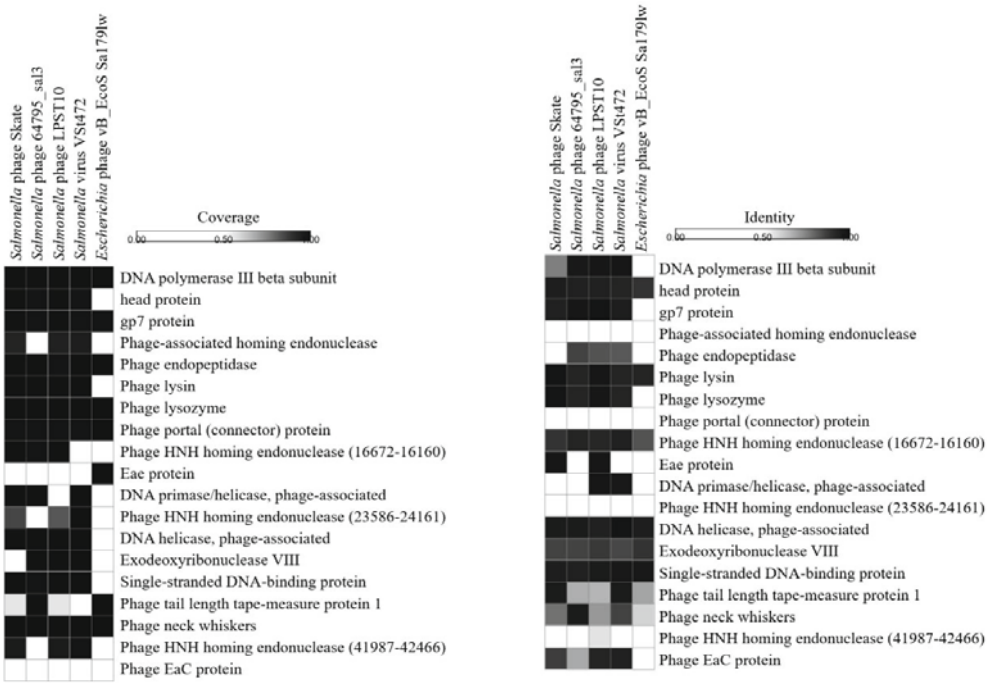

Fig. 4. KFS-SE2 phage protein comparison using BLASTP. The heatmap shows results for 19 annotated KFS-SE2 proteins with those of closely related phage strains. The color scales indicate the proportion of sequence coverage and identity.

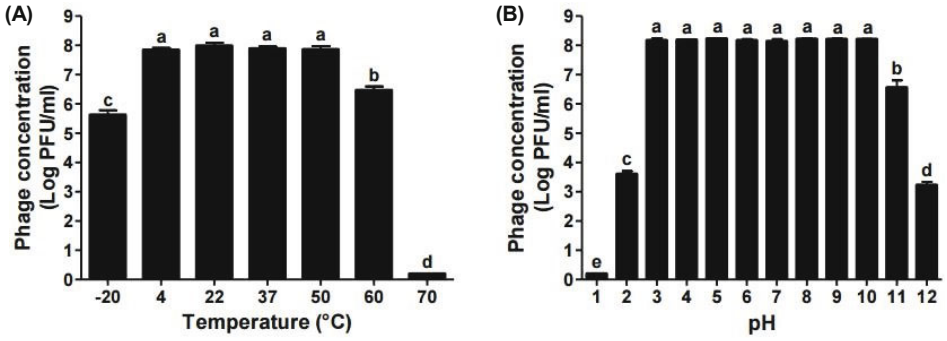

Fig. 5. Stability of KFS-SE2 phage under various (A) temperature and (B) pH conditions. Different letters (a–c) represent significant differences at $P < 0.05$. The data represents the mean ± SD (n = 3).

tity reveal that most of the unclassified proteins based on RAST subsystem show low coverage and identity with those of closely related phage strains (Fig. 4); EaC and EaE proteins were quite different features among relative strains. Also, 2 of 3 HNH homing endonuclease proteins are less conserved among these phage strains. Phage-associated homing endonuclease and phage portal (connector) proteins show high coverage but low identity.

Specificity of KFS-SE2 phage

Consideration of phage specificity is essential for its use as a new bio-receptor to ensure the reliability of biosensor method. The majority of foodborne pathogens associated with fresh produces outbreaks were used in this study. The specificity of KFS-SE2 phage was investigated against 14 *Salmonella* strains and 23 non-*Salmonella* strains. As shown in Table 1, KFS-SE2 phage exhibited excellent specificity to *S*. Enteritidis among 14 *Salmonella* strains and 23 non-*Salmonella* strains.

Stability of KFS-SE2 phage

A phage-employed sensor may be exposed to various environmental conditions (temperatures and pHs) for pathogen detection because fresh produces are exposed to various harsh conditions during their processing and storage. Therefore, phage needs to sustain its activity under these harsh and unfavorable conditions. As shown in Fig. 5A, KFS-SE2 phage was stable at 4–50°C whereas its stability was significantly decreased at -20°C and 60°C by approximately 29% and 19%, respectively ($P < 0.05$). At a further harsh temperature condition (70°C), phage stability was lost up to approximately 97%. Meanwhile, the pH stability of KFS-SE2 phage (Fig. 5B) was well sustained at pH ranges of 3–10. Then, the phage stability was significantly decreased starting from pH 11 (approximately 19% reduction) with further reductions at pH 2 (approximately 56% reduction) and pH 12 (approximately 60% reduction), followed by almost complete loss at pH 1 (approximately 98% reduction) ($P < 0.05$).

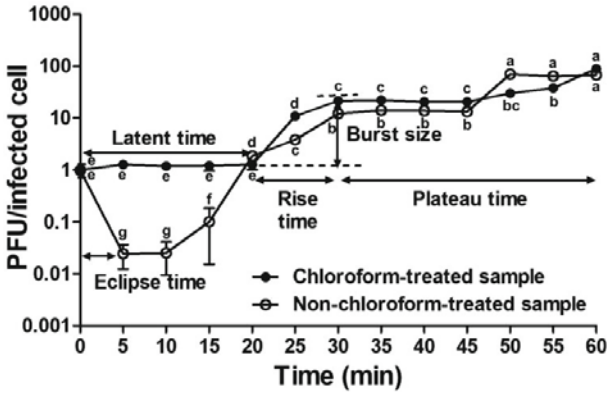

Fig. 6. One-step growth curve of KFS-SE2 phage. Open circles (○), non-chloroform-treated sample and closed circles (●), 1% chloroform-treated sample. Different letters (a–g) represent significant differences among incubation time within the same treatment at $P < 0.05$. The data represents the mean ± SD (n = 3).

Measurement of latent time of KFS-SE2 phage

Since KFS-SE2 phage had a lytic property, the phage can invade the host and integrate its DNA for replication in the host and eventually release the lysis of their host (Young, 2014; Gwak *et al.*, 2018). The lysis of bacterial cell on the mass-based sensor surface will cause less resonant frequency shifts due to weight loss of bacteria. The detection of phage immobilized on the sensor with target pathogen needs to be done prior to the lysis of bacterial cell. Thus, the latent time (the time interval between the adsorption of phage into host cell and the beginning of the first burst out from the host) should be considered when employing lytic phage on the mass-based biosensor method (Li and Zhang, 2014; Byeon *et al.*, 2015). Phage lytic cycle consists of eclipse time (time between infection by phage and the appearance of mature virus within the host cell), latent time (mentioned above), rise time (time for increasing the number of lysed phage) and plateau time (time for sustaining the number of lysed phage) (Wang, 2006), as shown in Fig. 6. The latent time of KFS-SE2 phage was determined to be 20 min, indicating the beginning of first burst out of phage from the host. In addition, the burst size of KFS-SE2 phage was determined to be 20 PFU/cell by calculation of phage yields that lysed from one infected cell.

Discussion

Up to now, most phage studies have been focused on its isolation, purification, and characterization as a new bio-control agent rather than as a bio-receptor. Based on previous studies, 23 *Salmonella*-specific phages have been reported and classified into 12 *Siphoviridae*, 10 *Myoviridae*, and 2

Podoviridae family based on morphological characteristics (Carey-Smith *et al.*, 2006; De Lappe *et al.*, 2009; Turner *et al.*, 2012; Tiwari *et al.*, 2013). From the morphological observation (Fig. 1), KFS-SE2 phage had similar head and tail lengths with other *Siphoviridae* phages such as SE2 infecting *S.* Enteritidis (Tiwari *et al.*, 2013), SETP 12 infecting *S.* Enteritidis (De Lappe *et al.*, 2009), vB_SenS-Ent1infecting *Salmonella* (Turner *et al.*, 2012), and FGCSSa2 infecting *S.* Typhimurium (Carey-Smith *et al.*, 2006).

KFS-ES2 phage had a relatively lower GC content of 45.8% than that of *S.* Enteritidis genomes (52%) and 2 tRNA genes (tRNA-Met-CAT and tRNA-Arg-TCT; Fig. 2), which may overcome possible differences in codon usage between the host and the phage (Edwards *et al.*, 2002; Bailly-Bechet *et al.*, 2007; Thomson *et al.*, 2008). KFS-SE2 phage may replicate faster due to optimal translation by tRNAs (Bailly-Bechet *et al.*, 2007; Amarillas *et al.*, 2017). In addition, the lytic property of KFS-SE2 phage was confirmed, because there are no encoding genes for lysogenic property (Fig. 2). Moreover, it could be safe for employment as a bio-receptor due to the absence of encoding genes associated with virulence factor, antibiotic resistance, and potential allergen (Fig. 2). PhageTerm predicts the KFS-SE2 genome is permuted with terminal redundancy (Garneau *et al.*, 2017), which is characteristic of a head-full packaging strategy (Casjens and Gilcrease, 2009). These results clearly indicate that KFS-SE2 phage has many distinctive characteristics as a new phage based on genome analysis.

The phylogenetic analysis confirmed that KFS-SE2 phage belongs to genus *Pis4avirus* within *Siphoviridae* (Fig. 3). Comparative analysis (Fig. 4) of annotated KFS-SE2 proteins with closely related strains based on the phylogenetic analysis clearly showed that KFS-SE2 phage has some distinct proteins associated with DNA modification (homing endonucleases) (Stoddard, 2011) and DNA packaging (portal protein) (Casjens *et al.*, 1992), which may suggest that KFS-SE2 may have unique features with other closely related phages. However, EaE and EaC proteins have been used as *Salmonella* phage markers despite their functions being unknown (Wullf *et al.*, 1993; Bardina *et al.*, 2016; Proroga *et al.*, 2018). Moreover, there are many unannotated proteins (71 out of 90 proteins) and no clear evidence of functional proteins related to phage binding with *S.* Enteritidis (Fig. 2). Further studies are necessary to understand the functional features of KFS-SE2 phage in detail.

Since food can be contaminated with other foodborne pathogens, the specificity of bio-receptor is a very critical factor to identify a target pathogen only among other contaminated microorganisms in foods (Park *et al.*, 2012a; Byeon *et al.*, 2015). The excellent specificity of KFS-SE2 phage was confirmed by cross-examination with other major foodborne pathogens associated with fresh produces (Yeni *et al.*, 2016; Oh and Park, 2017). KFS-SE2 phage exhibited excellent specificity to *S.* Enteritidis only among 14 *Salmonella* strains and 23 non-*Salmonella* strains (Table 1). Carey-Smith *et al.* (2006) reported that FGCSSa1 infecting *Salmonella* exhibited a broad specificity over six *Salmonella* strains including *S.* Enteritidis, *S.* Typhimurium, and *S.* Saintpaul among eight *Salmonella* strains although it did not infect two *Escherichia coli* strains. ϕSTIz1 phage infecting *S.* Typhimurium also showed broad specificity over *S.* Typhimurium, *S.* Gallinarum, *S.* Abortus equi, *S.* Pollorum, and *S.* Enteritidis among a total of 14 strains including *S.* Virchow, *Staphylococcus aureus*, and *Escherichia coli* (Kerketta *et al.*, 2014). Compared with other studies (Carey-Smith *et al.*, 2006; Bao *et al.*, 2011; Tiwari *et al.*, 2013; Kerketta *et al.*, 2014; Kim *et al.*, 2018), KFS-SE2 phage exhibited specificity to *S.* Enteritidis. This will offer excellent advantages for our phage to detect *S.* Enteritidis only, even when fresh produces are contaminated with other pathogens.

Although KFS-SE2 phage is highly specific, the binding of phage with *S.* Enteritidis could be affected by several factors including temperature, pH, ionic strength in solution, incubation time as well as concentrations of KFS-SE2 and *S.* Enteritidis (Park *et al.*, 2011; Choi *et al.*, 2018). Among the various external factors that influence the binding between a phage and target, temperature and pH are dominant because phage can be precipitated, denatured, mutated, and dissociated (especially capsid protein) under harsh temperatures and pH conditions (Jończyk *et al.*, 2011). Furthermore, generally reported conditions of fresh produces range from pH 3 to 7 as well as a temperature range from 4°C to 22°C (Bridges and Mattice, 1939; Waisnawa *et al.*, 2018). Due to this reason, when a phage employed sensor is exposed to various temperatures and pHs, its stability also needs to be sustained. Phage stability was measured as phage concentration at each condition (Fig. 5). The excellent stability of KFS-SE2 phage over the temperatures (4–50°C) and pHs (3–10) will provide a great advantage when employed. Therefore, this study confirmed the relatively excellent stability of KFS-SE2 phage, which will ensure practicability and reliability of our phage in field use.

Phage has two contradictory characteristics such as lysogenic (invade into their host genome and exist as plasmids within host) and lytic properties (Oh and Park, 2017; Gwak *et al.*, 2018). The best strategy for searching for an ideal phage from the environment as a new bio-receptor is to find lysogenic phages. Unfortunately, 96% of phages isolated from the environment are tailed phages so it is not easy to find the lysogenic phage despite the ubiquitous existence of phage (10^{31}–10^{32}) (Hosseinidoust *et al.*, 2014; Oh and Park, 2017). Hence, when employing lytic phage as a bio-receptor, the total detection time should be completed before host lysis due to the action of lytic phage. Furthermore, since our detection system is based on the subtle mass change on the sensor surface, the lysis of host cell on the sensor surface will decrease total weight of mass attached on the sensor, resulting in less changes of resonant frequency shift. From the result of Fig. 6, the latent time was determined to be 20 min with a burst size of 20 PFU/infected cell, which was even closer to that of fmb-p1 phage [20 min, 77 PFU/infected cell, (Wang *et al.*, 2017)] and PSPu-95 phage [20 min, 77.5 PFU/infected cell, (Bao *et al.*, 2011)], and shorter than that of SFP10 phage [25 min, 200 PFU/infected cell, (Park *et al.*, 2012b)], ΦSP-1 phage [30 min, 44 PFU/infected cell, (Augustine *et al.*, 2013)] and SPN9CC phage [30 min, 220 PFU/infected cell, (Shin *et al.*, 2014)]. This limitation will be overcome by controlling total incubation time of phage with bacteria. Based on our preliminary study using a mass-based surface scanning detection system (data not provided), the signal of resonant

frequency shift was observed starting from 5 min and saturated at 15 min once phage-immobilized sensor was placed on the *S.* Enteritidis-contaminated food surface. In addition, the latent time of phage at natural environments could be extended rather than at laboratory-level optimum condition (Mao *et al.*, 2009). Therefore, 20 min is sufficient time for KFS-SE2 phage to interact with *S.* Enteritidis without any negative effect of lytic phage. Therefore, a new lytic phage isolated from the environment was investigated as a potential bio-receptor in this study. Overall, the highly specific, safe, economic, and relatively stable KFS-SE2 phage could be a good candidate as a new bio-receptor and employed to our mass-based biosensor method to detect *S.* Enteritidis on fresh produces.

Acknowledgements

This research was funded by the Basic Research Program through the National Research Foundation (NRF) of Korea founded by the Ministry of Education (NRF-2017R1D1A1-B03035195).

Conflict of Interest

There are no conflict of interests.

References

Amarillas, L., Rubí-Rangel, L., Chaidez, C., González-Robles, A., Lightbourn-Rojas, L., and León-Félix, J. 2017. Isolation and characterization of phiLLS, a novel phage with potential biocontrol agent against multidrug-resistant *Escherichia coli*. *Front. Microbiol.* **8**, 01355.

Arndt, D., Grant, J.R., Marcu, A., Sajed, T., Pon, A., Liang, Y., and Wishart, D.S. 2016. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res.* **44**, W16–W21.

Arya, S.K., Singh, A., Naidoo, R., Wu, P., McDermott, M.T., and Evoy, S. 2011. Chemically immobilized T4-bacteriophage for specific *Escherichia coli* detection using surface plasmon resonance. *Analyst* **136**, 486–492.

Augustine, J., Louis, L., Varghese, S.M., Bhat, S.G., and Kishore, A. 2013. Isolation and partial characterization of ΦSP-1, a *Salmonella* specific lytic phage from intestinal content of broiler chicken. *J. Basic Microbiol.* **53**, 111–120.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M., *et al.* 2008. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* **9**, 75.

Bailly-Bechet, M., Vergassola, M., and Rocha, E. 2007. Causes for the intriguing presence of tRNAs in phages. *Genome Res.* **17**, 1486–1495.

Bao, H., Zhang, H., and Wang, R. 2011. Isolation and characterization of bacteriophages of *Salmonella enterica* serovar Pullorum. *Poult. Sci.* **90**, 2370–2377.

Bardina, C., Colom, J., Spricigo, D.A., Otero, J., Sánchez-Osuna, M., Cortés, P., and Llagostera, M. 2016. Genomics of three new bacteriophages useful in the biocontrol of *Salmonella*. *Front. Microbiol.* **7**, 00545.

Batz, M.B., Hoffmann, S., and Morris, J.G.Jr. 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *J. Food Prot.* **75**, 1278–1291.

Bridges, M.A. and Mattice, M.R. 1939. Over two thousand estimations of the pH of representative foods. *Am. J. Dig. Dis.* **6**, 440–449.

Bushnell, B. 2014. BBMap. Available from https://sourceforge.net/projects/bbmap/ (accessed on Nov. 06, 2018).

Byeon, H.M., Vodyanoy, V.J., Oh, J.H., Kwon, J.H., and Park, M.K. 2015. Lytic phage-based magnetoelastic biosensors for on-site detection of methicillin-resistant *Staphylococcus aureus* on spinach leaves. *J. Electrochem. Soc.* **162**, B230–B235.

Carey-Smith, G.V., Billington, C., Cornelius, A.J., Hudson, J.A., and Heinemann, J.A. 2006. Isolation and characterization of bacteriophages infecting *Salmonella* spp. *FEMS Microbiol. Lett.* **258**, 182–186.

Casjens, S.R. and Gilcrease, E.B. 2009. Determining DNA packaging strategy by analysis of the termini of the chromosomes in tailed-bacteriophage virions. *Methods Mol. Biol.* **502**, 91–111.

Casjens, S., Wyckoff, E., Hayden, M., Sampson, L., Eppler, K., Randall, S., Moreno, E.T., and Serwer, P. 1992. Bacteriophage P22 portal protein is part of the gauge that regulates packing density of intravirion DNA. *J. Mol. Biol.* **224**, 1055–1074.

Chen, L., Zheng, D., Liu, B., Yang, J., and Jin, Q. 2016. VFDB 2016: hierarchical and refined dataset for big data analysis–10 years on. *Nucleic Acids Res.* **44**, D694–D697.

Choi, I.Y., Lee, J.H., Kim, H.J., and Park, M.K. 2017. Isolation and characterization of a novel broad-host-range bacteriophage infecting *Salmonella enterica* subsp. *enterica* for biocontrol and rapid detection. *J. Microbiol. Biotechnol.* **27**, 2151–2155.

Choi, I.Y., Park, J.H., Gwak, K.M., Kim, K.P., Oh, J.H., and Park, M.K. 2018. Studies on lytic, tailed *Bacillus cereus*-specific phage for use in a ferromagnetoelastic biosensor as a novel recognition element. *J. Microbiol. Biotechnol.* **28**, 87–94.

De Lappe, N., Doran, G., O'connor, J., O'hare, C., and Cormican, M. 2009. Characterization of bacteriophages used in the *Salmonella enterica* serovar Enteritidis phage-typing scheme. *J. Med. Microbiol.* **58**, 86–93.

Doyle, M.P. and Buchanan, R.L. 2013. Food microbiology: Fundamentals and frontiers, 4th ed, pp. 187–236. American Society for Microbiology, Washington, D.C., USA.

Edwards, R.A., Olsen, G.J., and Maloy, S.R. 2002. Comparative genomics of closely related salmonellae. *Trends Microbiol.* **10**, 94–99.

Farris, J.S. 1974. Formal definitions of paraphyly and polyphyly. *Syst. Zool.* **23**, 548–554.

Garneau, J.R., Depardieu, F., Fortier, L.C., Bikard, D., and Monot, M. 2017. PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data. *Sci. Rep.* **7**, 8292.

Gervais, L., Gel, M., Allain, B., Tolba, M., Brovko, L., Zourob, M., Mandeville, R., Griffiths, M., and Evoy, S. 2007. Immobilization of biotinylated bacteriophages on biosensor surfaces. *Sensor Actuat. B-Chem.* **125**, 615–621.

Göker, M., García-Blázquez, G., Voglmayr, H., Tellería, M.T., and Martín, M.P. 2009. Molecular taxonomy of phytopathogenic fungi: a case study in *Peronospora*. *PLoS One* **4**, e6319.

Gwak, K., Choi, I., Lee, J., Oh, J., and Park, M. 2018. Isolation and characterization of a lytic and highly specific phage against *Yersinia enterocolitica* as a novel biocontrol agent. *J. Microbiol. Biotechnol.* DOI: 10.4014/jmb.1808.08001.

Hiremath, N., Guntupalli, R., Vodyanoy, V., Chin, B.A., and Park, M.K. 2015. Detection of methicillin-resistant *Staphylococcus aureus* using novel lytic phage-based magnetoelastic biosensors. *Sensor Actuat. B-Chem.* **210**, 129–136.

Hosseinidoust, Z., Olsson, A.L., and Tufenkji, N. 2014. Going viral: designing bioactive surfaces with bacteriophage. *Colloids Surf. B: Biointerfaces* **124**, 2–16.

Hungaro, H.M., Mendonça, R.C.S., Gouvêa, D.M., Vanetti, M.C.D.,

and de Oliveira Pinto, C.L. 2013. Use of bacteriophages to reduce *Salmonella* in chicken skin in comparison with chemical agents. *Food Res. Int.* **52**, 75–81.

Jończyk, E., Kłak, M., Międzybrodzki, R., and Górski, A. 2011. The influence of external factors on bacteriophages. *Folia Microbiol. (Praha)* **56**, 191–200.

Kerketta, P., Agarwal, R., Rawat, M., Jain, L., Kumar, P.P., Dhanze, H., Kumar, M.S., and Kumar, A. 2014. Isolation and characterization of lytic bacteriophage (ϕSTIz1) against *Salmonella enterica* serovars Typhimurium. *J. Pure Appl. Microbiol.* **8**, 4719–4726.

Kim, S., Kim, S.H., Rahman, M., and Kim, J. 2018. Characterization of a *Salmonella* Enteritidis bacteriophage showing broad lytic activity against Gram-negative enteric bacteria. *J. Microbiol.* **56**, 917–925.

Lefkowitz, E.J., Dempsey, D.M., Hendrickson, R.C., Orton, R.J., Siddell, S.G., and Smith, D.B. 2017. Virus taxonomy: the database of the international committee on taxonomy of viruses (ICTV). *Nucleic Acids Res.* **46**, D708–D717.

Lefort, V., Desper, R., and Gascuel, O. 2015. FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. *Mol. Biol. Evol.* **32**, 2798–2800.

Li, S., Horikawa, S., Shen, W., and Chin, B. 2010. Detection of *Salmonella* Typhimurium on fresh food produce using phage-based magnetoelastic biosensors. *ECS Trans.* **33**, 91–97.

Li, L. and Zhang, Z. 2014. Isolation and characterization of a virulent bacteriophage SPW specific for *Staphylococcus aureus* isolated from bovine mastitis of lactating dairy cattle. *Mol. Biol. Rep.* **41**, 5829–5838.

Mao, C., Liu, A., and Cao, B. 2009. Virus-based chemical and biological sensing. *Angew. Chem. Int. Ed. Engl.* **48**, 6790–6810.

McArthur, A.G., Waglechner, N., Nizam, F., Yan, A., Azad, M.A., Baylay, A.J., Bhullar, K., Canova, M.J., De Pascale, G., and Ejim, L., *et al.* 2013. The comprehensive antibiotic resistance database. *Antimicrob. Agents Chemother.* **57**, 3348–3357.

Meier-Kolthoff, J.P. and Göker, M. 2017. VICTOR: genome-based phylogeny and classification of prokaryotic viruses. *Bioinformatics* **33**, 3396–3404.

Meier-Kolthoff, J.P., Hahnke, R.L., Petersen, J., Scheuner, C., Michael, V., Fiebig, A., Rohde, C., Rohde, M., Fartmann, B., and Goodwin, L.A. 2014. Complete genome sequence of DSM 30083^{T}, the type strain (U5/41^{T}) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Stand. Genomic Sci.* **9**, 2.

Oh, J.H. and Park, M.K. 2017. Recent trends in *Salmonella* outbreaks and emerging technology for biocontrol of *Salmonella* using phages in foods: a review. *J. Microbiol. Biotechnol.* **27**, 2075–2088.

Olsen, E.V., Sorokulova, I.B., Petrenko, V.A., Chen, I.H., Barbaree, J.M., and Vodyanoy, V.J. 2006. Affinity-selected filamentous bacteriophage as a probe for acoustic wave biodetectors of *Salmonella* Typhimurium. *Biosens. Bioelectron.* **21**, 1434–1442.

Park, M.K. and Chin, B.A. 2016. Novel approach of a phage-based magnetoelastic biosensor for the detection of *Salmonella enterica* serovar Typhimurium in soil. *J. Microbiol. Biotechnol.* **26**, 2051–2059.

Park, M.K., Hirematha, N., Weerakoon, K.A., Vaglenov, K.A., Barbaree, J.M., and Chin, B.A. 2013a. Effects of surface morphologies of fresh produce on the performance of phage-based magnetoelastic biosensors. *J. Electrochem. Soc.* **160**, B6–B12.

Park, M., Lee, J.H., Shin, H., Kim, M., Choi, J., Kang, D.H., Heu, S., and Ryu, S. 2012b. 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.

Park, M.K., Li, S., and Chin, B.A. 2013b. Detection of *Salmonella* Typhimurium grown directly on tomato surface using phage-based magnetoelastic biosensors. *Food Bioprocess Technol.* **6**, 682–689.

Park, M.K., Oh, J.H., and Chin, B.A. 2011. The effect of incubation temperature on the binding of *Salmonella* Typhimurium to phage-based magnetoelastic biosensors. *Sensor Actuat. B-Chem.* **160**, 1427–1433.

Park, M.K., Wikle, H.C., Chai, Y., Horikawa, S., Shen, W., and Chin, B.A. 2012a. The effect of incubation time for *Salmonella* Typhimurium binding to phage-based magnetoelastic biosensors. *Food Control* **26**, 539–545.

Proroga, Y.T., Capuano, F., Capparelli, R., Bilei, S., Bernardo, M., Cocco, M.P., Campagnuolo, R., and Pasquale, V. 2018. Characterization of non-typhoidal *Salmonella enterica* strains of human origin in central and southern Italy. *Ital. J. Food Saf.* **7**, 6888.

Putturu, R., Eevuri, T., Ch, B., and Nelapati, K. 2015. *Salmonella* Enteritidis-food borne pathogen-a review. *Int. J. Pharm. Biol. Sci.* **5**, 86–95.

Rambaut, A. 2017. FigTree-version 1.4.3, a graphical viewer of phylogenetic trees. Available from http://tree.bio.ed.ac.uk/software/figtree/ (accessed on Nov. 06, 2018).

Sharma, H. and Mutharasan, R. 2013. Review of biosensors for foodborne pathogens and toxins. *Sensor Actuat. B-Chem.* **183**, 535–549.

Shin, H., Lee, J.H., Yoon, H., Kang, D.H., and Ryu, S. 2014. Genomic investigation of lysogen formation and host lysis systems of the *Salmonella* temperate bacteriophage SPN9CC. *Appl. Environ. Microbiol.* **80**, 374–384.

Sillankorva, S.M., Oliveira, H., and Azeredo, J. 2012. Bacteriophages and their role in food safety. *Int. J. Microbiol.* **2012**, 863945.

Singh, A., Arutyunov, D., Szymanski, C.M., and Evoy, S. 2012. Bacteriophage based probes for pathogen detection. *Analyst* **137**, 3405–3421.

Singh, A., Glass, N., Tolba, M., Brovko, L., Griffiths, M., and Evoy, S. 2009. Immobilization of bacteriophages on gold surfaces for the specific capture of pathogens. *Biosens. Bioelectron.* **24**, 3645–3651.

Singh, A., Poshtiban, S., and Evoy, S. 2013. Recent advances in bacteriophage based biosensors for food-borne pathogen detection. *Sensors* **13**, 1763–1786.

Sorokulova, I., Olsen, E., and Vodyanoy, V. 2014. Bacteriophage biosensors for antibiotic-resistant bacteria. *Expert Rev. Med. Devices* **11**, 175–186.

Stoddard, B.L. 2011. Homing endonucleases: from microbial genetic invaders to reagents for targeted DNA modification. *Structure* **19**, 7–15.

Sun, W., Brovko, L., and Griffiths, M. 2001. Use of bioluminescent *Salmonella* for assessing the efficiency of constructed phage-based biosorbent. *J. Ind. Microbiol. Biotechnol.* **27**, 126–128.

Thomson, N.R., Clayton, D.J., Windhorst, D., Vernikos, G., Davidson, S., Churcher, C., Quail, M.A., Stevens, M., Jones, M.A., and Watson, M. 2008. Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. *Genome Res.* **18**, 1624–1637.

Tiwari, B.R., Kim, S., and Kim, J. 2013. A virulent *Salmonella enterica* serovar Enteritidis phage SE2 with a strong bacteriolytic activity of planktonic and biofilmed cells. *J. Bacteriol. Virol.* **43**, 186–194.

Turner, D., Hezwani, M., Nelson, S., Salisbury, V., and Reynolds, D. 2012. Characterization of the *Salmonella* bacteriophage vB_SenS-Ent1. *J. Gen. Virol.* **93**, 2046–2056.

USDA. 2013. Foodborne illness cost calculator: *Salmonella*. Available from http://www.ers.usda.gov/topics/food-safety/foodborne-illness/salmintro.asp (accessed on Oct. 30, 2018).

Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K., and Adley, C. 2010. An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnol. Adv.* **28**, 232–254.

Waisnawa, I., Santosa, I., Sunu, I., and Wirajati, I. 2018. Model development of cold chains for fresh fruits and vegetables distribution: a case study in bali province. *J. Phys. Conf. Ser.* **953**, 012109.

Wang, N. 2006. Lysis timing and bacteriophage fitness. *Genetics*

172, 17–26.

Wang, C., Chen, Q., Zhang, C., Yang, J., Lu, Z., Lu, F., and Bie, X. 2017. Characterization of a broad host-spectrum virulent *Salmonella* bacteriophage fmb-p1 and its application on duck meat. *Virus Res.* **236**, 14–23.

Wullf, D.L., Ho, Y.S., Powers, S., and Rosenberg, M. 1993. The *int* genes of bacteriophages P22 and λ are regulated by different mechanisms. *Mol. Microbiol.* **9**, 261–271.

Yeni, F., Yavaş, S., Alpas, H., and Soyer, Y. 2016. Most common food-borne pathogens and mycotoxins on fresh produce: a review of recent outbreaks. *Crit. Rev. Food Sci. Nutr.* **56**, 1532–1544.

Young, R. 2014. Phage lysis: three steps, three choices, one outcome. *J. Microbiol.* **52**, 243–258.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., and Larsen, M.V. 2012. Identification of acquired antimicrobial resistance genes. *J. Antimicrob. Chemother.* **67**, 2640–2644.