Isolation, characterization, and genome investigation of vB_SenS_TUMS_E4, a polyvalent bacteriophage against *Salmonella enteritidis*

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Abstract

Background

Salmonellosis is a critical and common infectious malady between people and animals caused by Salmonella bacteria. With the advent of antibiotic resistance, it is essential for new methods to be replaced to prevent and treat infections. Bacteriophages are promising choices.

Methods

In this study, phage vB_SenS_TUMS_E4 against *Salmonella enteritidis* isolate has been separated from poultry wastewater. Determination of phage characteristics, including plaque formation, imaging with transmission electron microscopy, growth curve, structural proteins profile, host range, and pH and temperature parameters. Also, the Phage genome was extracted, sequenced, and annotated, and by utilizing Average Nucleotide Identity and phylogeny was compared with reference *Salmonella* phages.

Results

The burst size was large, nearly 287 plaque-forming units per cell (PFU/cell) and elevated stability to many temperatures and pH values. Phage vB_SenS_TUMS_E4 was effective on various clinical and environmental strains of *Salmonella* but did not affect bacteria of other genera. The morphological analysis indicated that phage vB_SenS_TUMS_E4 belongs to the *Siphoviridae* family. The genome of vB_SenS_TUMS_E4 is a linear dsDNA molecule of 43,018 bp with a G + C content of 49.7%. It includes 60 protein-coding genes, yet it contains no tRNA genes. Among the 60 detected putative protein-coding genes, just 43 gene products were contained in database searches. No genes associated with antibiotic resistance, virulence factor, and lysogenic were realized in the vB_SenS_TUMS_E4 genome.

Conclusion

The findings indicate that the high lytic potency vB_SenS_TUMS_E4 polyvalent phage is an antibacterial agent for controlling Salmonella in food production, prevention, and Salmonella treatment.

Introduction

Salmonella is one of the four leading causes of diarrhea worldwide (1). *Salmonella* bacteria are among the zoonotic bacteria that infect humans and animals. Poultry products are the primary source of *Salmonella* through food (2). *Salmonella enterica* serovar *enteritidis*, in addition to being responsible for infection in poultry neighbors and worsening economic hardships in poultry industry, also infects humans via consumption of contaminated meat and eggs (3). The emergence of antibiotic resistance has become a major global health concern (4, 5). Some resistant *Salmonella* serotypes have emerged and

have affected the food chain (2). Nowadays, the conventional pathogen anti-contamination protocols in food processing facilities focus mainly on using chemicals, physical disturbance techniques, and radiation to reduce the microbial load. However, no single approach is 100% effective, and the mentioned approaches have significant disadvantages. Consequently, the recent trend has been towards identifying non-chemical and environmentally friendly alternative antimicrobial strategies (known as green) (6). Therefore, one of the strategies to control *Salmonella* in the food chain is the application of bacteriophages (2). Contamination prevention and safe food supply are high-priority issues for controlling and limiting the prevalence of foodborne pathogens under a "one health" approach (6). Using bacteriophages against food pathogens such as *Salmonella* to improve the food industry has received more attention. For that reason, most of the regulatory approvals are related to phage products of food pathogens(7).

Bacteriophages (or phages) are obligate parasites that specifically infect bacteria (4). They could be used as bio-preservatives in food production, and as an agent to detect pathogenic bacteria in the food systems (8–10). Desirable properties of the bacteriophages such as specific binding to the target host, self-replication and self-limiting properties (11), no receptor on eukaryotic cells (12), lack of impact on normal flora (5, 13), and the elimination of the antibiotic-resistant bacteria as a strategy have been proposed to remove pathogens from the food chain as well as other environments. Because the mechanisms of bacterial resistance to phages are different from those of antibiotics, the strains that develop resistance to antibiotics usually remain sensitive and can be killed by bacteriophages. Thus, phages may help manage various bacterial infections, including infections caused by drug-resistant bacterial strains (6). An essential point in phage therapy is the preparation of a lytic phage library without antibiotic resistance genes, toxin encoding genes, or any other harmful factors for humans and animals (14). This study isolated a new lytic bacteriophage from poultry wastewater which was examined for genetic and biological.

Material And Methods

Bacterial strains and growth conditions

As phage screening host, *Salmonella enteritidis* ATCC13076 was achieved from the Department of Microbiology, Faculty of Veterinary Medicine, Tehran University. Bacterial strains used in this study were collected from different centers and kept at -70 °C in Luria Bertani broth (LBB; Liofilchem®, Italy) including 15% (v/v) glycerol as a cryoprotectant.

Isolation, propagation and preparation of phages

Poultry solid sewage samples collected from broiler and laying hen farms in Lorestan, Iran, were used for phage isolation. All solid specimens were subjected to centrifugation at 10,000 × g for 10 min for the removal of large particles followed by filtering through a 0.22 µm filter (Millipore, Ireland) to discard bacterial cells and small debris [13](15). Regarding phage enrichment, 5 mL of filtrate was added to LBB (10 mL) and *Salmonella enteritidis* ATCC 13076 (2.5 mL) with Optical density (OD) = 0.5. After incubation

of the mixture overnight at 37 °C, it was subjected to centrifugation and filtration. The filtrate was assessed for the existence of phages through spotting 5 µL into double-layer agar that contained *S. enteritidis* bacterial lawn. The bacterial property was provided through the addition of pre-cultured bacteria *S. enteritidis* to LBA with 0.7% agar and it was poured into pre-solidified Luria-Bertani Agar (LBA) plates. For purifying the phages, a micropipette tip was used to pick isolated plaques, then suspended in an exponential phase *Salmonella* culture for 24 h at 37 °C. Centrifugation (8000 × g for 15 min) of the culture was done, and it was filtered on 0.22 µm filters, and applied as the single phage culture [5, 14]. Repeated single plaque isolation was used to purify phage plaques using sterile micropipette tips for at least three rounds to achieve pure phage stocks [15], and supplies were kept at 4 °C until further use.

Plaque morphology analysis

The plaque morphology assessment of bacteriophages was done simultaneously with the PFU assay. We prepared ten-fold dilutions of phage lysate in LBB; 100 µL of overnight host culture was added to appropriate proper dilution of phage lysate (100 µL) and mixed with 4 mL of LB with 0.5% agar. Plates containing 20 mL of LB agar were added with the mixture. The double-layer agar plates were subjected to incubation at 37 ° for 24 h. Plaque diameter and morphology were assessed.

Transmission electron microscopy (TEM) of vB-SenS-TUMS-E4

Transmission electron microscopy was used for observation and characterization of phage morphology. Ultra-centrifugation of the freshly prepared pure phage suspension (10^{10} PFU/ml) was done at 40,000×g, 4 ∘C for 1 h (SIGMA 3-30KS refrigerated centrifuge) for sedimentation of phage particles. After discarding the supernatant, the pellet was washed for two times using 0.1 M ammonium acetate solution. 200 µl of the specimen was stained using 1% uranyl acetate and sprayed on formaldehyde copper grade and dried in air (16). A TEM was used to observe stained phage (Phillips, EM208S, Netherlands) operating at 100 kV.

Structural protein profiles

To measure the phage structural protein profile, s SDS polyacrylamide gel electrophoresis (SDS-PAGE) of the ultra-centrifuged phage particle was done. About 10^{12} phage particles were suspended in SDS-PAGE loading buffer (10 % glycerol, 2 % SDS, 0.0025 % bromophenol blue, 2.5 % β-mercaptoethanol, and 6.25 mM Tris/HCl, pH 6.8), followed by boiling for 10 min, and loading into a 12 % polyacrylamide gel. Pre-stained protein ladder (11 - 245 kDa) (Cinacolon, Iran) was applied as a standard PAGE ruler. Following electrophoresis, staining of the gel was done using Coomassie brilliant blue R250 (Merck) for 5 min, followed by destaining with destaining solution (10 % acetic acid and 30 % methanol in distilled water), and photographing on a light box (17).

The host range

The spot test for phage E4 was performed on *Salmonella enterica* serovars and other gram-negative and gram-positive species for determining the hosts range of the isolated phage. Phage effectiveness was assessed through spotting pure phage suspension (10 µl), fixed to 10^8 PFU/ml, into a lawn of potential bacterial hosts. Following incubation at 37 °C for 16–18 h, the plates were assessed for host inhibition sites (plaques) (18).

One-step growth of phage vB-SenS-TUMS-E4

The latent period as well as the average burst size were calculated through one-step growth test according to the modified method by Clokie MR et al. (2009)(19). Briefly, 0.1 ml of the prepared phage (MOI 0.001) was added to 9.9 ml of bacterial culture medium that contained 0.1 M calcium chloride and incubated for 5 min at 37 °C. After 5 min, 0.1 mL was mixed with 9.9 mL of fresh pre-warmed medium. The medium was diluted twice and sampled from 0 to 90 min at appropriate dilutions.

Time-kill analysis of *Salmonella enteritidis* treated with phage

The time-kill assay was performed to determine the susceptibility of plankton bacterial cells to phage infection and evaluate the abundance of phage-resistant bacteria. Using a 1:10 diluted bacterial culture of OD_{600}= 0.5, the multiplicities of infection (MOI) of 0.01, 0.1, 1, 10, and 100 PFU/CFU were anticipated in comparison with uninfected bacterial control (phage-free samples) at 37 °C. The sampling time points were 0, 2, 4, 6, 8, and 24 h, and the samples were diluted and spread into LB plates for determination of the CFU (20).

Temperature and pH Stability Assay

Phage stability was evaluated under various temperatures and pH. To evaluate the temperature stability, we incubated the phage suspension (10^{10} PFU/ml) at various temperature values of 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C for 90 min. Regarding the pH stability, the phage suspension was mixed with LB medium with various pH of 3, 4, 5, 6, 7, 8, and 9 adjusted with 1 M NaOH and 1 M HCl solutions. Sampling was performed every 10 min. Phage particles count was measured by preparing dilution from phage suspension and drop test (21).

DNA genome extraction and restriction analyses

DNase (Fermentas Life Sciences, UK) and proteinase K (Norgen Biotek Corp., Thorold, ON, Canada) were used to treat the high-titer phage suspension. Then phage DNA isolation kit (Norgen Biotek Corp., Thorold, ON, Canada) was used to extract phage nucleic acid as instructed. The concentration and quality of the extracted nucleic acid were determined with a Nano drop spectrophotometer. The DNA of E4 bacteriophage was digested using the restriction enzymes EcoRI (Fermentas), and HindIII (Fermentas), following the product instructions, then the DNA fragments were assessed through electrophoresis on 1% agarose gel in 1x TAE buffer for 40 min at 120 V (22).

Sequencing and Genome Assembly

The DNA library was constructed by the Library NEBNext® Ultra™ IIDNA Library Prep kit, and high-throughput sequencing was done by the Illumina Novaseq PE150 platform (San Diego, CA, USA). The obtained fragments were in paired-end reads with lengths of 150 bp. FASTQC (Version 0.72+galaxy1) (23) and Trimmomatic (Version 0.38.0) (3) were used for quality control and trimming of the reads and adapters, leading to more than 8 million paired-end sequence reads. Subsampling of the reads was performed with seqtk (Galaxy Version 1.3.2) (24). De novo assembly was conducted with the Shovill pipeline (Version 3.12.0) using default parameters (7). Assembly validation was performed in Galaxy BWA-MEM (24) and Blastn was used to identify similarities between the assembled genome and other genomic sequences. Genomic termination was determined using PhageTerm (Version 3.4.0 CPT Galaxy https://cpt.tamu.edu/galaxy-pub). Since the PhageTerm identified no specific genomic terminals, the genome formed a circular set. PCR and Sanger sequencing of the contig ends (forward primer, 5'-AAGTAGAGGAACCTGCACC-3' and reverse primer, 5'-AAGTACGAAACACCATGCAC-3') were used for closing and verification of the genome sequence (25). The complete genome sequence of *Salmonella* phage vB_SenS_TUMS_E4 was cached in the National Center for Biotechnology Information (NCBI) repository under the accession number of MZ955866.

Genome Sequence Analysis

Rapid Annotation of phage was performed with RAST pipeline, and then Blastp was used manually to confirm annotations (21). tRNAscan-SE v2.0, PhagePromoter (CPT Galaxy), ARNOLD (http://rssf.i2bc.paris-saclay.fr/toolbox/arnold/), and phage AI servers (3) were applied to search tRNA, predict promoters, Rho-factor independent terminators, and lifestyle (23). The Virulence Factor Database (VFDB) and Antibiotic Resistance Genes Database (ARDB)were used to identify antibiotic resistance genes as well as virulence factors (22, 26). Whole-genome sequence comparisons of E4 and *Salmonella* phage 5sent1 with accession number MT653144; bacteriophages were formed using BLASTn and observed using Easyfig software(26, 27).

Phylogeny

To measure the average nucleotide identity (ANI), some similar phage genomes in *Jerseyvirus* were downloaded from the NCBI through the ANIb (according to BLAST calculation) in the JSpeciesWS Web Server (http://jspecies.ribohost.com) (28). Regarding phylogenetic assessment, the genome sequences related to the *jersey* phages were achieved from the GenBank database and aligned with MAFFT. Mesquite software was used to edit the alignment file. Phylogenetic assessment was done according to the complete genome of phages. Phylogenetic trees were constructed in IQ-TREE server through the maximum likelihood approach with 1000 bootstrap replications. The tree was visualized using Figtree.

Results

Morphology of Phage vB_SenS_TUMS_E4

Phage E4 produces large, transparent, round plaques, 1.5 to 2 mm in size and slightly turbid halos on isolated *Salmonella enteritidis* host cultures (Fig.1a). It is suggested that halo formation results from endolysin secretion after the lysis of the host cells (29). According to Phage morphological analysis by transmitting electron microscopy, the phage exhibited a hexagonal capsid with a diameter of 82±2 nm and a non-contractile tail with a length of 150±5 nm (Fig.1b). Morphological assessment of phages indicated that they belonged to the *Siphoviridae* family.

Host range properties of the phage

Analysis of the host domain of phage E4 showed that this phage lyses all strains of *Salmonella*, including some drug-resistant strains (Table 1). At the same time, E4 did not show lytic ability on strains of other genera. Host specificity as a prerequisite for phage therapy is applied in the food industry, particularly for fermented foods or the gut microbiota in farm animals. These results indicate the high specificity of the vB_SenS_TUMS_E4.

Table 1
Host range of phage vB_SenS_TUMS_E4.

No	Species	strainID	Lytic Activity
1	*Salmonella enteritidis* ATCC 13076	ATCC 13076	+
2	*Salmonella typhi* ATCC 14023	ATCC 14023	+
3	*Salmonella Paratyphi*	Lab Collection	+
4	*Salmonella entrica*	Lab Collection	+
5	*Salmonella typhi*	Lab Collection	+
6	*Salmonella Paratyphi* C	Lab Collection	+
7	*Salmonella* clinical	Lab Collection	+
8	*Salmonella* clinical	Lab Collection	+
9	*Salmonella* clinical	Lab Collection	+
10	*Salmonella* clinical	Lab Collection	+
11	*Escherichia coli* PTCC 1330	PTCC 1330	-
12	*Escherichia coli* ATCC 25922	ATCC 25922	-
13	*Staphylococcus aureus* ATCC 6588	ATCC 6588	-
14	*Staphylococcus aureus* ATCC 23591	ATCC 23591	-
15	*Staphylococcus aureus* ATCC 33591	ATCC 33591	-
16	*Staphylococcus aureus* ATCC 25923	ATCC 25923	-
17	*Pseudomonas syringae* ATCC 10205	ATCC 10205	-
18	*Pseudomonas aeruginosa* ATCC 9027	ATCC 9027	-
19	*Pseudomonas aeruginosa* ATCC 27853	ATCC 27853	-
20	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Lab Collection	-
21	*Staphylococcus saprophyticus*	Lab Collection	-
22	*Proteus mirabilis*	Lab Collection	-
23	*Enterococcus faecalis*	Lab Collection	-
24	*Serratia marcescens*	Lab Collection	-
25	*Pseudomonas putida*	Lab Collection	-
26	*Enterobacter aerogenes*	Lab Collection	-
27	*Citrobacter freundi*	Lab Collection	-

No	Species	strainID	Lytic Activity
28	*Shigella flexneri*	Lab Collection	-
29	*Citrobacter freundii*	Lab Collection	-
30	*Staphylococcus epidermidis*	Lab Collection	-
31	*Acinetobacter baumannii*	Lab Collection	-
32	*Klebsiella pneumoniae*	Lab Collection	-
33	*Lactobacillus salivarius*	Lab Collection	-
34	*Lactobacillus jensenii*	Lab Collection	-
35	*Lactobacillus plantarum*	Lab Collection	-
36	*Lactobacillus reuteri*	Lab Collection	-

One-step growth of phage vB-SenS-TUMS-E4

A one-step growth test determined the latent period as well as phage size of vB_SenS_TUMS_E4. The latent period was about 15 min, and the burst size was approximately 287 plaque-forming units (PFU) per cell (Fig. 2a). The phage burst size is highly associated with its proliferation, and a right burst size is a favorable feature for an effective lytic bacteriophage. Thus, phages characterized by large burst size and short latency are suitable candidates for phage therapy (30). Hence, the found burst size and the short latency of VB_SenS_TUMS_E4 can be regarded as favorable features for its use in phage therapy.

Time-kill analysis of *Salmonella enteritidis* treated with phage

The phage lytic activity was assessed against *S. enteritidis* culture at its exponential developmental phase (OD600=0.4) with various MOIs of E4. After 2 h, using MOIs of 0.01, 0.1, 1, 10, and 100 led to a nearly 3.5 Log dcrease in the bacterium titer to approximately 10^2 CFU/ml ($P<0.0001$). Phage titers were similar at various MOIs, suggesting that the E4 phage titer was less affected by MOI. Nevertheless, lower MOIs (0.1 and 0.01) caused a moderate reduction in the bacterium titer in early hours (Fig. 2b). Thus, the application of higher phage levels led to a faster decrease in the bacterium count, possibly because of the elevated attachment rate at a higher phage titer. Also, incubation for 24 h significantly increased in the bacterium titer in both phage-treated and control samples. The recorded bacterium titers were about 2 Log less compared to the control ($P<0.0001$). Therefore, the observed decrease in the host bacterium cell count within the first three hours of being exposed to E4 phage and preserving such trend up to 8 h following incubation can be a marked feature for the possible use of this phage in phage therapy.

Temperature and pH Stability

Using phages as biocontrol agents to inhibit bacteria requires their stability under different stress conditions, like pH and temperature. pH stability testing of the E4 indicated that it is highly stable at pH 5 to 9 (P<0.0001). Nevertheless, under acidic conditions (pH=3), after 90 min, 4 logs of phage titer were observed (Fig. 2c).

Fig. 2d shows phage E4 is relatively stable at high temperatures without significant titer loss up to 50 °C following incubation for 90 min (P<0.0001). After incubation of the phage at 60 °C, PFU/mL decreased 2 logs in titer. Exposing phage E4 at temperatures more than 70 °C caused rapid inactivation and titer reductions of 6 log PFU/ml.

Restriction profile

The DNA of phage E4 could be digested by both HindIII and EcoRI (Fig. 3), which indicated that the nucleic acid contained in phage E4 is double-stranded DNA.

vB_SenS_TUMS_E4 genome characterization

We obtained a complete assembled phage genome of vB_SenS_TUMS_E4 (43018 bp) and 49.7% C+G content from genome sequencing and annotation. The PhageTerm and PCR analysis results showed that the genome is circularly permuted (data not shown)(31). Based on RAST annotation analysis, a total of 60 protein encoding genes were predicted for E4, of which 38 genes are on the positive strand, and 22 genes are on the negative strand (Fig. 4). About 89% of genes start with ATG, and 11% with GTG (E4-2, 9, 49, and 54) and TTG (E4-5, 28, and 33). Three types of end codons were used to predict ORFs, including TAA (53%, 32 ORFs), TGA (33%, 20 ORFs), and TAG (13%, 8 ORFs). Functional analysis using Blastp revealed 43 genes encoding proteins with known functions and 17 genes with unknown functions (Additional File 1: Table S1). The vB_SenS_TUMS_E4 genome can be divided into four modules based on the predicted functions of its component genes: (i) phage assembly and structure, (ii) DNA packaging, (iii) replication and regulation proteins (iv) host lysis (Fig. 5). In addition, 21 transcriptional promoter sequences were recognized using Phage Promoter software (Additional File 1: Table S2). Thirty-seven Rho factor independent terminators were determined in the E4 genome by Arnold (Additional File 1: Table S3), which were evaluated based on the existence of a U-rich tail, their location, and strongly anticipated stem-loop secondary structure ($\Delta G \leq -10$ kcal/mol) determined using MFold (32). The completeness of the phage E4 was confirmed after mapping (99.94%) the reads with BWA-MEM (24). Our analysis showed that the E4 phage lacked known genes that encode antibiotic resistance, toxins, or virulence factors. Therefore, this phage is possibly a virulent phage against *Salmonella enteritidis*.

Phage structure and assembly

Based on Blastp bioinformatics analysis, the genome of Phage 4 contains 20 structural proteins. Fifteen genes related to tail and tail assembly (E4-3, 8, 14-21, 27-29, 33, and 34), five genes related to capsid and capsid assembly, and head morphogenesis were identified (E4-7, 11-13, and 46) (Additional File 1: Table

S1). The structural module occupies the positive strand of genome, 20 genes including structural proteins and those involved in virion assembly.

Nine protein bands presenting the virion structural components were observed in the SDS polyacrylamide Coomassie-stained gel (Fig. 6). A dominant polypeptide bond of about 37 kDa indicates the presence of the original capsid protein resulting in a high number of copies of capsid proteins. The molecular mass identified corresponds to the protein anticipated molecular weight (Fig. 6). Major capsid protein is a highly conserved protein. A polypeptide bond of about 72 kDa indicates the presence of the tail spike protein (Fig. 6).

DNA packaging

Above the head module is the E4 DNA packaging module. The packaging machine usually consists of a portal ring and a terminase complex in tailed phages (29, 30). The most characteristic terminases are hetero-oligomers consisting of a small subunit (E4-1) involved in DNA detection and a large terminal subunit (E4-2) containing ATPase and endonuclease activities. E4-2 encodes terminase large subunit protein, which shows more than 99% identity (with query coverage 100%) with terminase large subunit protein encoded by VSe103, SE2 (Additional File 1: Table S1).

Replication and regulation proteins

E4 genome possessed four core genes associated with phage replication, like DNA polymerase I (E4-41), homing endonuclease (E4-24, 38, and 45), helicase (E4-48), and exonuclease (E4-36). Based on the analysis using Blastp, three regulatory core genes were identified. The gene coding for helix-turn-helix for regulating gene expression (E4-26,48, and 51), the gene coding for putative NinH-like protein transcriptional regulation, and phage site-specific recombination (E4-59), and the gene coding for putative DNA-binding protein (E4-6), were found.

Lysis

Lysis genes are usually found near each other and form a lysis cassette. In some *siphoviridae* members, including vB_SenS_SE1, wks13, FSL-SP031, K1H, and E4, the endolysin genes are upstream of the packaging genes (Fig. 5) (23). E4-56 encodes lysin protein, which shows more than 93% identity (with query coverage of 100%) with endolysin encoded by VSe103, SE2, and Ent1.

Phylogeny analysis

The E4 phage genome was compared with already sequenced phages in GenBank to study the evolutionary relationship of phage. Then, the E4 genome and the phages belonging to the *Jerseyvirus* were analyzed with JSpeciesWS, and the results showed that the ANI values for phages were above 80% (Additional File 1: Table S4)(33). Thus, phage E4 can be a member of the *Jerseyvirus* genus. Phylogenetic analysis based on the complete genome showed that phage E4 is closely related to bacteriophage 5sent1

(Fig. 7) belonging to the genus of *Jerseyvirus*. These findings are in line with those of *Jersey* phages who indicated low sequence similarity to those in the *Siphoviridae* family(7, 34).

Statistical Analysis

Data are presented as mean ± standard deviation. We applied GraphPad Prism 8.0.1 for statistical analysis. Two-way ANOVA with multiple comparisons was employed for indicating differences between groups at < 0.0001.

Discussion

Given the increasing antibiotic resistance globally, phage therapy seems to be a promising tool to combat these pathogens (7). Because bacteriophages are widely distributed worldwide, they are easily isolated from the natural environment (32). This study isolated a new *Salmonella* phage vB_SenS_TUMS_E4 from a poultry effluent sample and determined its biological and genomic properties. Phage E4 belongs to the genus *Jerseyvirus* in the family *Siphoviridae*, and its G + C content, genome organization, and morphology are similar to those previously described phages of this genus. Approximately half of the genes encoding by *Jerseyvirinae* phages belonged to adult virions' genome assembly, packing, and structure. As it was observed for many comet phages, E4 genome shows a modular organization with functionally related genes (Fig. 4). The architecture of this module in E4 shows typical features of most *Siphoviruses*: The head and tail genes are found on the positive strand of DNA. According to most type 1 *Siphoviridae*, the head-tail joining protein gene (E4-15,16) is usually located at downstream of the major capsid protein gene (E4-12) (23). In E4 genome, E4-12 encodes major capsid protein, which shows more than 97% identity (with query coverage of 100%) with the capsid protein encoded by VSe103, and SE2. The tail spike allows the phage to attach to specific receptors on the surface of the host bacterium and infects host cells by recognition of the O antigenic repeating units on the cell surface (35). E4-34 encodes tail spike protein, which shows more than 95% identity (with 100% query coverage) with tail spike protein encoded by VSe103, and SE2. E4-33, the most prominent protein (852 amino acids) in the genome of vB_SenS_TUMS_E4, encodes a member of phage–tail superfamily protein and presents 99% identity to tail fiber protein of *Salmonella* virus VSe103, and 98.59% identity (with query coverage of 100%) with tail fiber protein of *Salmonella* phage SE2 (Additional File 1: Table S1). Terminase proteins are responsible for identifying DNA and initiating DNA packaging in all phages studied (28). The small subunit is responsible for DNA binding, and the large subunit terminase is responsible for binding and cleavage of the concatemers' phage DNA inward (36). E4-41 encodes DNA polymerase I (99.59% identity to DNA polymerase I of Escherichia phage buks, 99.45% identity to DNA polymerase I of *Salmonella* phage SETP13, and 98.77% identity to that of *Salmonella* phage vB_SenS_ER18). E4-45 encodes HNH endonuclease, showing 100% identity (with 100% query coverage) to HNH endonuclease of *Salmonella* phage vB_SenS_PVP-SE2 (Additional File 1: Table S1).

Lysine, holin, and Spanin are proteins that facilitate the lysis of the infected host cells and allow the newly formed virion particles to escape into the environment (37). Holin lysis the bacterial cell membrane,

thus exposing the cell wall to lysozyme, which breaks down bacterial peptidoglycans and releases phages (38, 39). Spanins are the third functional class of lysis proteins needed to disrupt the outer membrane in the final stage of gram-negative host lysis. The phage protein E4-10 appears to be 100% identity (with query coverage 100%) to the putative spanin of *Salmonella* phage celemicas (Additional File 1: Table S1). The phage protein E4-54 appears to be 95.7% identity (with 100% query coverage) to the putative holin of *Salmonella* phage *jersey*. Since all known *Jersey* members are entirely lytic (7, 40), phage E4 showed lytic activity and lacked genes associated with the lysogenic cycle. In addition, annotating the entire sequence of its genome does not confirm any virulence factor in its genome, including genes for resistance to toxins or antibiotics. Analysis of phage E4 genes indicated the existence of genes that encode home endonucleases that are functionally related to this phenomenon. Phage E4 showed a shorter latent length as well as a larger blast size compared to other *Jersey* phages. Since such factors are essential in the host lysis system (41), E4 can be considered a beneficial anti-*Salmonella* agent. Compared with reported studies, E4 has similar thermal and pH stability (22). Different strains of *Salmonella* were sensitive to this phage. However, bacteria of other genera, including some bacteria of human and poultry normal flora (42), did not show any sensitivity to this phage. Therefore, vB_SenS_TUMS_E4 can be introduced as a valuable tool in the biological control of salmonellosis.

Abbreviations

LB
Luria–Bertani
PFU
Plaque forming unit
MOI
Multiplicity of infection
TEM
Transmission electron microscopy
ANI
Average nucleotide identity
NCBI
National Center for Biotechnology Information
OD
Optical density

Declarations

Data availability statement

Our data set can be found in online repositories. Repository name/repositories and access number (s) can be found below: https://www.ncbi.nlm.nih.gov/nuccore/MZ955866.1/

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Author Contributions

ZS and AS conceived and designed research. NT and HK conducted experiments. ZS contributed new reagents or analytical tools. MK and NT analyzed data. NT wrote the manuscript. All authors read and approved the manuscript.

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Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare no conflict of interest.

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Figures

Figure 1

(a) Plaques of phage vB_SenS-TUMS E4 on double-layer agar plates of *S.enteritidis* isolate. (b) Transmission electron micrograph of the phage.

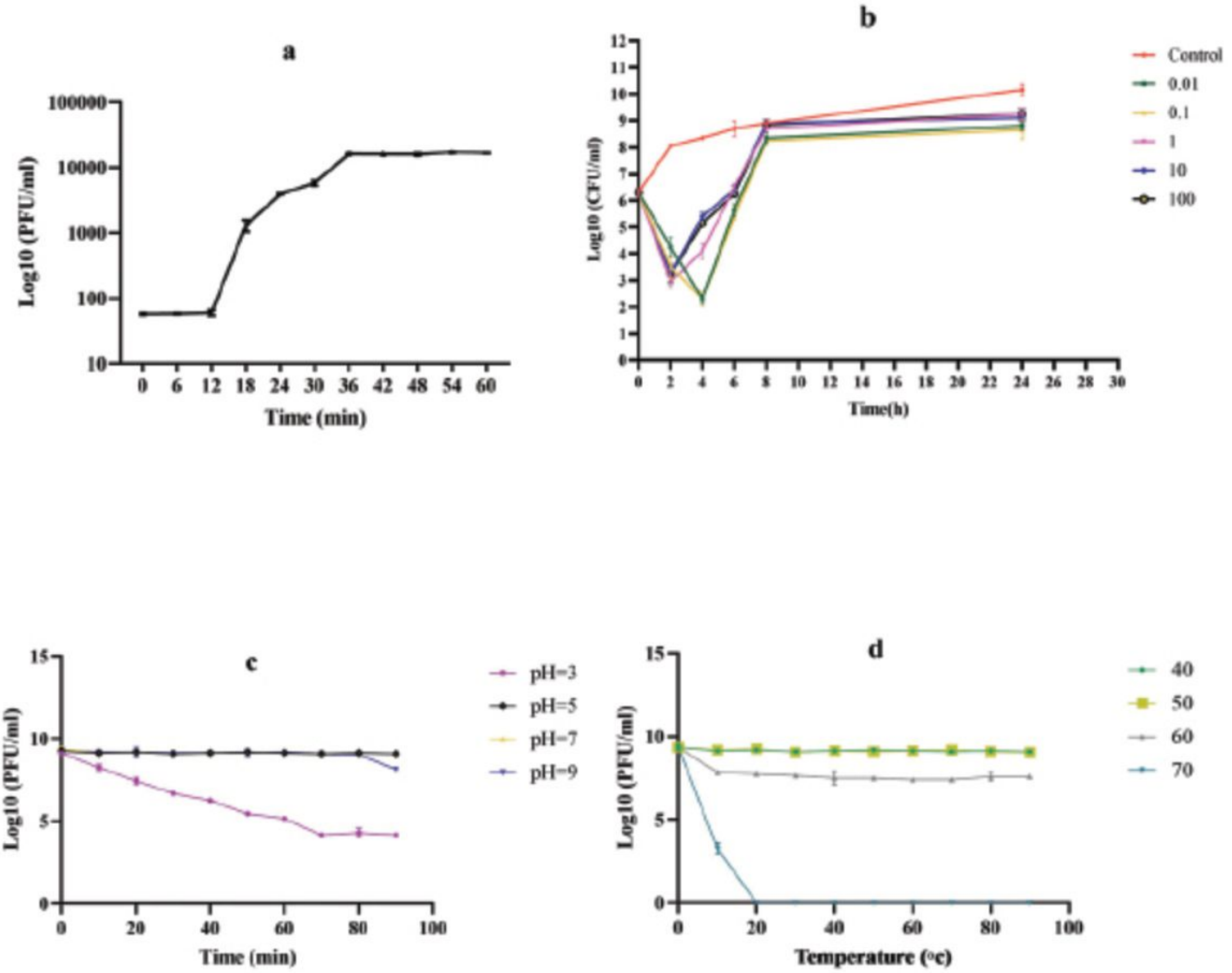

Figure 2

(a) One step growth curve of vB_SenS_TUMS_E4 (b) Time kill of vB_SenS_TUMS_E4 Stability of phage vB_SenS_TUMS_E4 (c) pH stability (d) Thermal stability.

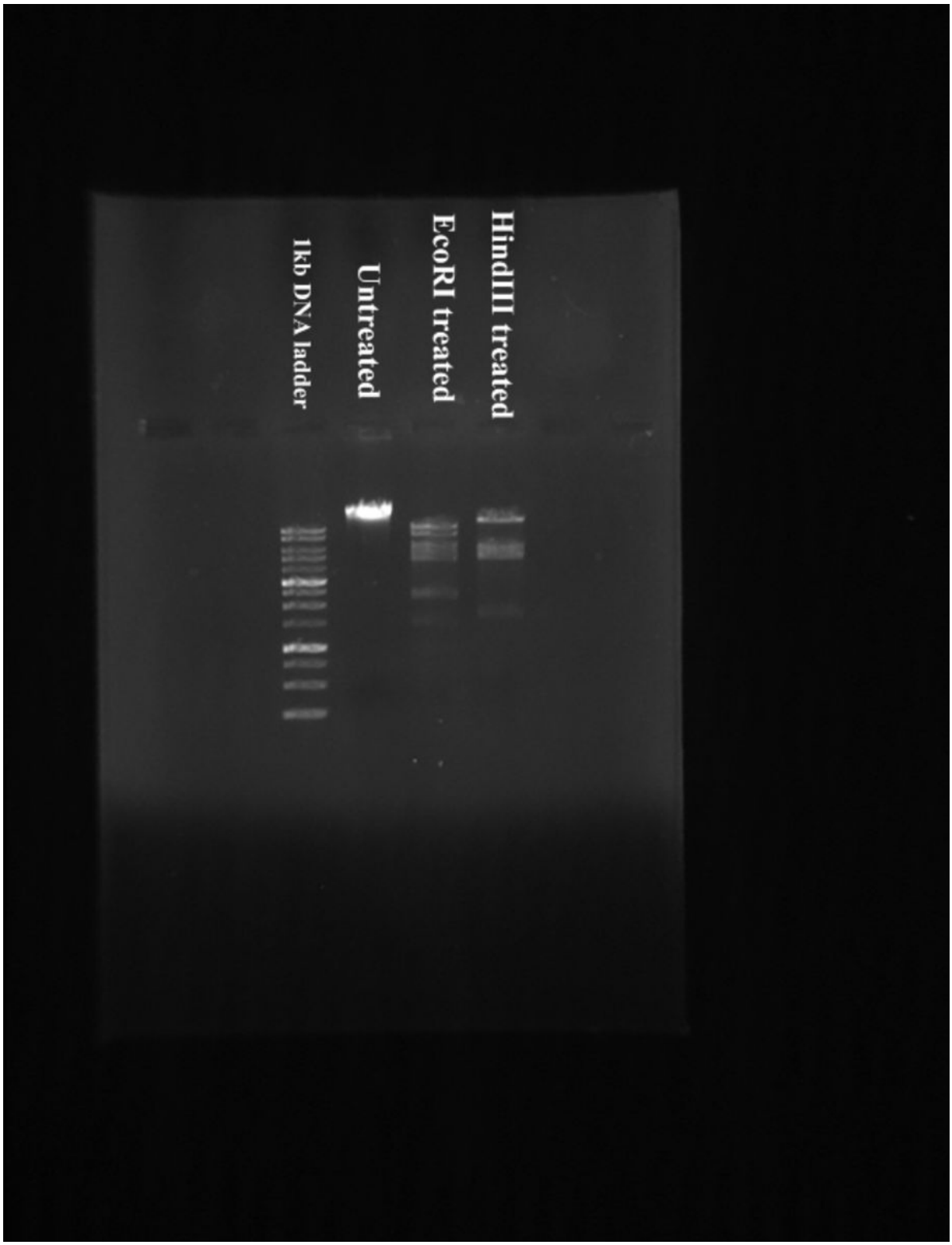

Figure 3

Restriction fragments analysis of the phage vB_SenS_TUMS_E4. Untreated phage genomic DNA (lane 1) and digested with restriction enzymes EcoRI (lane 2), HindIII (lane 3), lane M, 25 Kb DNA marker (Cinacolon, Iran).

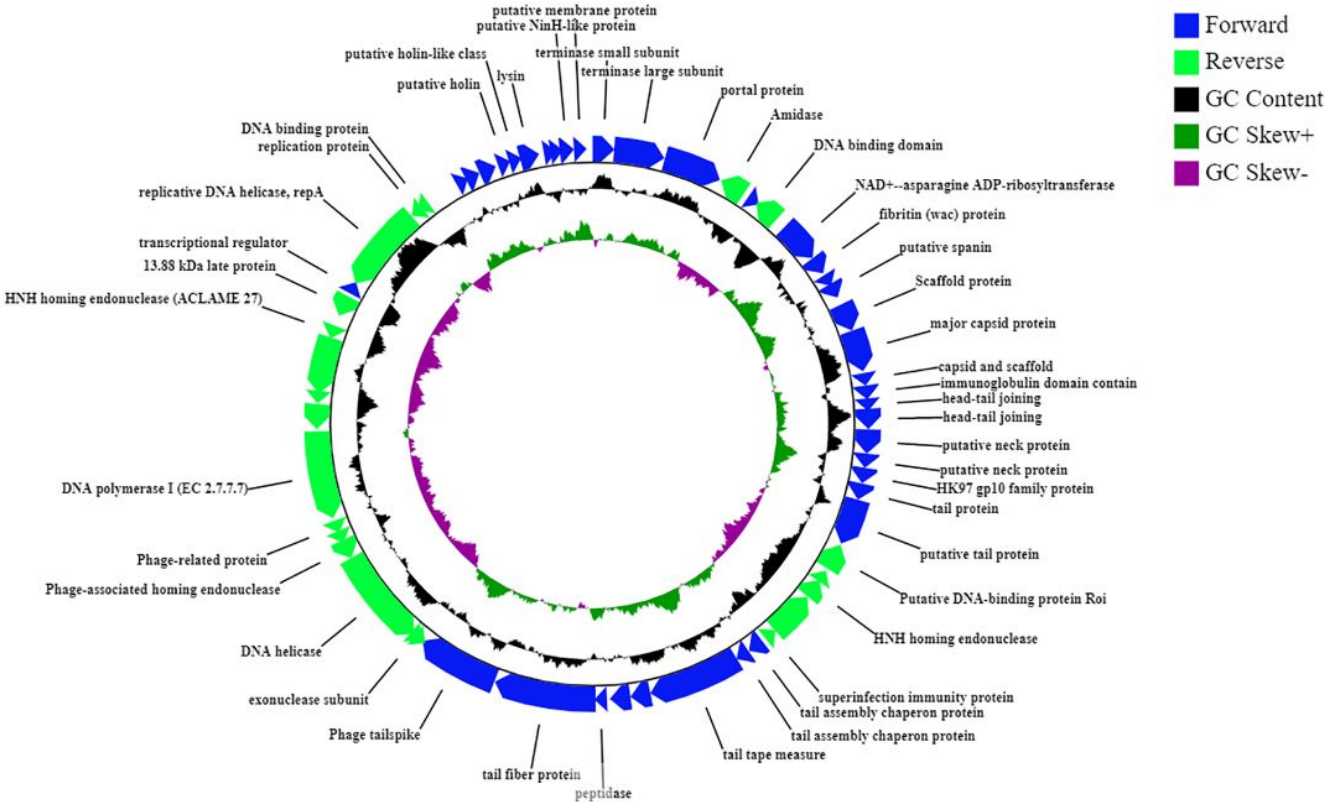

Figure 4

Physical and Genetic map of *Salmonella* phage vB_SenS_TUMS_E4 prepared using CGView (43). Blue indicates genes on the positive strand, and green indicates genes on the negative strand.

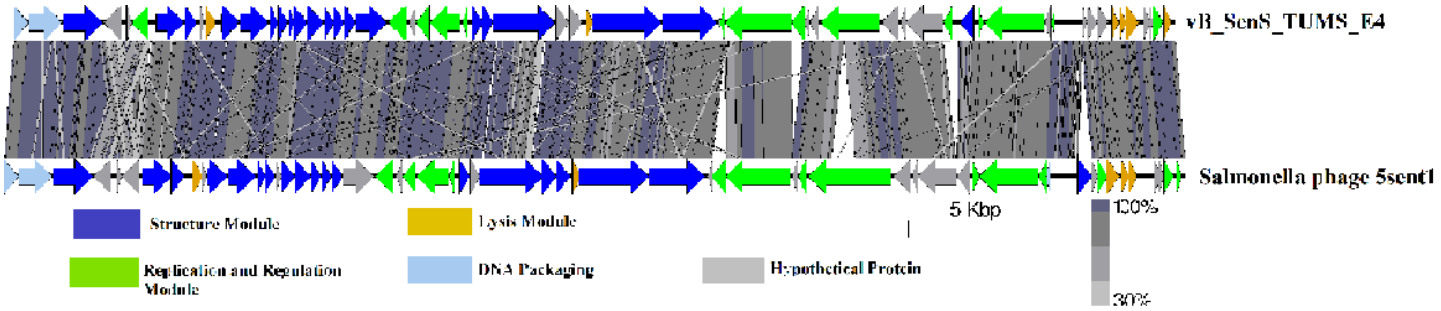

Figure 5

Genomic comparison of vB_SenS_TUMS_E4 and 5sent1 generated using Easyfig. Different colors denote genes with other functions. The similarities can be seen as gray lines, and the gray shading indicates the level of identification.

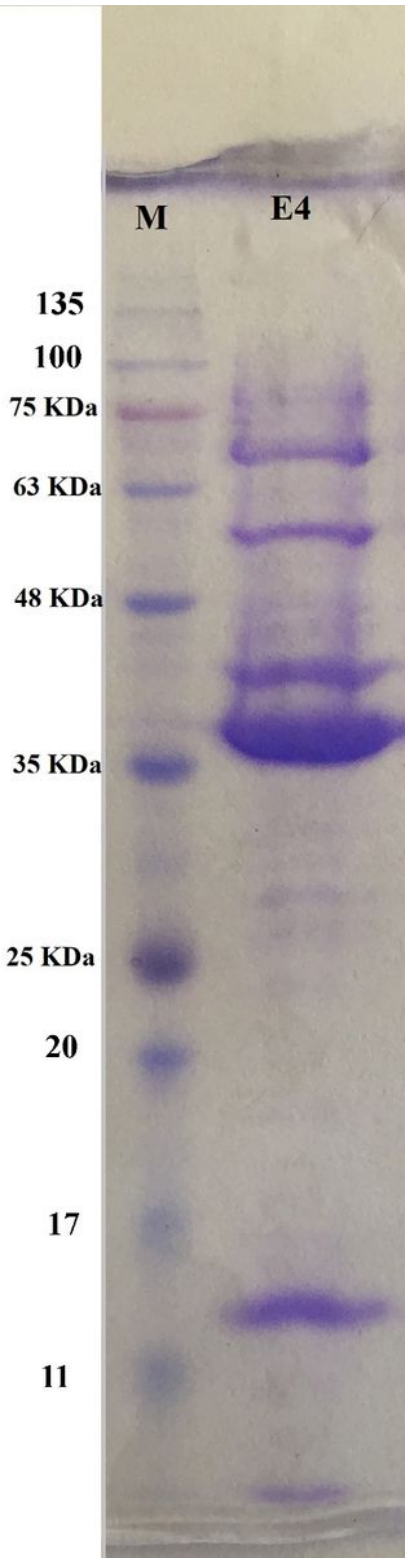

Figure 6

The SDS-PAGE analysis of phage VB_SenS_TUMS_E4 structural proteins on 12% gel staining using Coomassie brilliant blue. M, a standard marker of molecular weight (kDa).

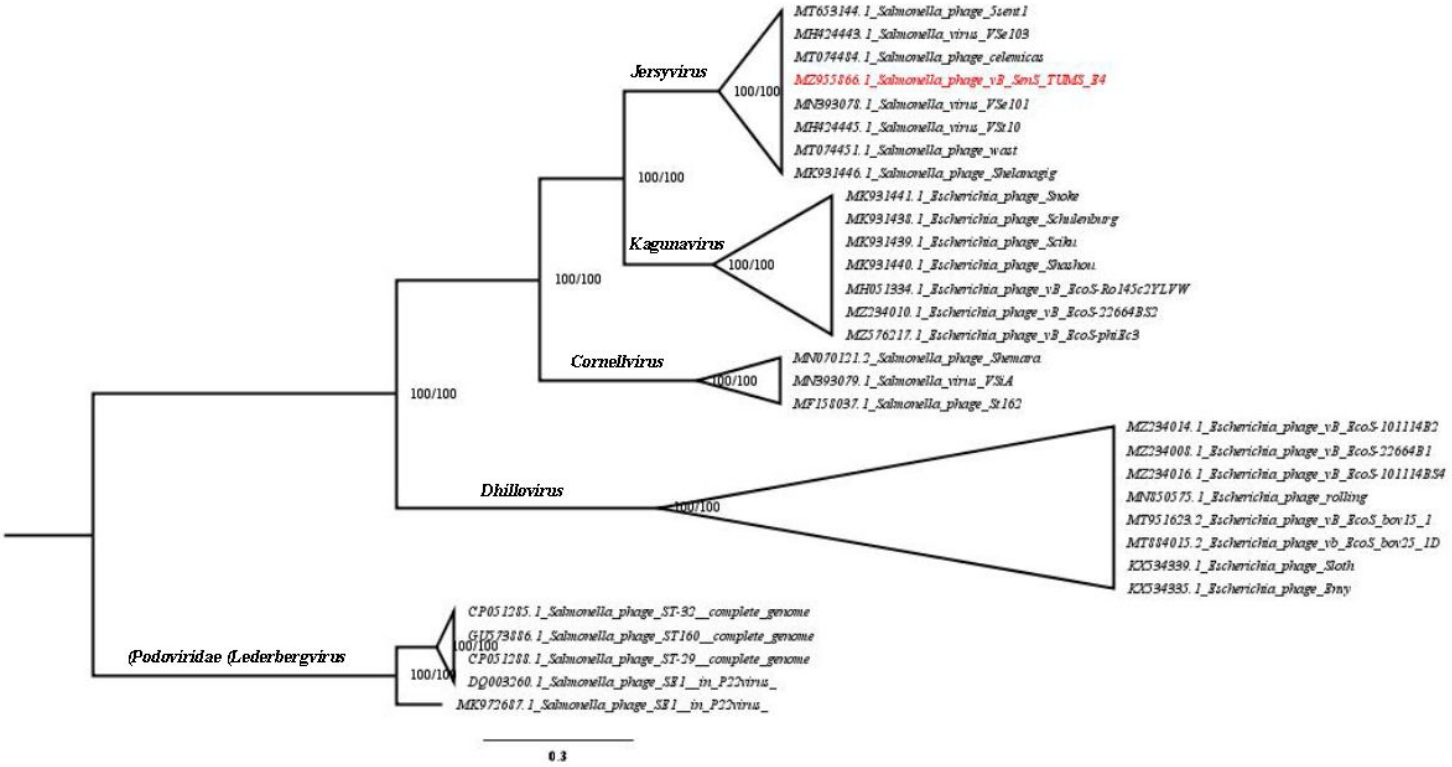

Figure 7

Phylogenetic assessment according to the complete genomes of bacteriophages showed that phage E4 belongs to the genus of *Jerseyvirus*.

Supplementary Files

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