Original Article

Whole-genome sequencing of *Salmonella* phage vB_SenS_TUMS_E15 for bio-control in the food chain

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ABSTRACT

The genome analysis of bacteriophages is crucial for their successful application in clinical and biocontrol settings. In this study, we isolated a new lytic phage, vB_SenS_TUMS-E15, from hospital sewage that was effective against *Salmonella enteritidis*, and analyzed its genomic features. The complete genome analysis revealed that E15 had circularly permuted double-stranded DNA of 43,048 base pairs (bp), with a G+C content of 49.7%. Sixty coding sequences (CDSs) were predicted in the genome, with 44 CDSs encoding known proteins in different modules, including packaging, structure, replication, metabolism, and lysis modules. No tRNA genes were found in the genome. Eight transcriptional promoter sequences and 37 rho-independent terminators were detected in the E15 genome. Phylogenetic analysis based on whole-genome sequences suggested that phage E15 should be classified as a member of the *Jersyvirus* genus in the subfamily *Guernseyvirinae*. Furthermore, no antibiotic-resistance genes, toxins, virulence factors, or lysogen-forming genes were observed in the genome. This suggests that E15 is a lytic phage, making it a promising candidate for clinical and biocontrol purposes.

Keywords: Bacteriophage, Salmonella enteritidis, Jersyvirus, Guernseyvirinae, Biocontrol

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Introduction

Salmonella enterica is a zoonotic bacterium that causes infectious diseases in humans and animals. It is a rod-shaped, gram-negative bacterium that belongs to the Enterobacteriaceae family. *Salmonella enterica* serovar Enteritidis is a significant foodborne pathogen that causes Salmonellosis and is associated with poultry and poultry products (1). The existence of antibiotic-resistant *Salmonella* strains poses a significant threat to public health, as it can lead to infections and outbreaks that are difficult to treat (2). Therefore, it is crucial to develop and implement novel strategies for the prevention and management of these infections. Phage therapy has been suggested as an alternative approach to combat bacterial infections, especially those caused by multidrug-resistant bacteria.

Bacteriophages are viral agents that can multiply only in the presence of their host and naturally decrease in

number in the absence of their host (3). Phages offer benefits in terms of therapy and food safety due to their low toxicity, absence of cross-resistance with antibiotics, and adaptable nature (4). Bacteriophages have been used as a targeted therapy for bacterial infections. Phages can specifically infect and kill pathogenic bacteria, making them a potential alternative to antibiotics, particularly for antibiotic-resistant bacteria (5). Also, bacteriophages can be used to control bacteria in the food industry. They can be applied to fruits, vegetables, meats, and other food products to kill or reduce the levels of harmful bacteria such as *Salmonella* or *E. coli*. This can help enhance food safety and reduce the risk of foodborne illnesses (6). Before selecting a phage for therapeutic or biotechnological purposes, it is crucial to evaluate its genomic and biological life cycles. Advancements in genome sequencing methods and bioinformatics software have enabled a more thorough understanding of bacteriophages, specifically in terms of their detection, safety, and efficacy (7). Phage E15 is a lytic virus that can infect various *Salmonella* serovars, making it an effective biocontrol agent for the food industry. Therefore, genome sequencing of E15 is crucial for understanding the genetic makeup of this phage and its potential for use in phage therapy.

Materials and Methods

Growth condition of *S. enteritidis*

S. enteritidis ATCC13076 was cultivated on Luria-Bertani agar (LB; Liofilchem®, Italy) plates at 37 °C for 18-24 hours. A single colony was then transferred to 10 ml of LB broth to create liquid cultures, which were incubated at 37 °C for 18-24 hours. To store the strains for future use, they were preserved at -80 °C in LB broth containing 15% (v/v) glycerol. This strain was obtained from the Department of Microbiology, Faculty of Veterinary Medicine, Tehran University.

Isolation, Enrichment and Purification of Phage E15

The E15 phage was obtained by adding raw hospital sewage (Tehran, Iran) to *S. enteritidis* ATCC 13067 during the early exponential phase for 24 hours at 37°C while shaking at 150 rpm. The mixture was then filtered using a low-protein-binding PES membrane filter with a porosity of 0.22 µm (Membrane Solution, USA). Spot tests were employed to verify the presence of phages, and samples that produced a clear zone were subsequently streaked on a culture plate to isolate individual plaques. The lytic phage was selected based on the presence of clear plaques on *S. enteritidis*, and a single plaque was purified using three repeats of the double-layer agar method according to previous protocols (8).

DNA extraction

To digest the exogenous DNA, the purified phage sample was treated with DNase I. Following this step, the enzyme was inactivated using heat, and the resulting mixture was exposed to proteinase K to break down the protective outer layer of the phage. The phage genomic DNA was extracted using a phage DNA isolation kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturer's instructions. The quality and concentration of the extracted DNA were assessed with electrophoresis on a 1% agarose gel and a Nanodrop spectrophotometer.

Sequencing and bioinformatics analysis of the E15 genome

The extracted genome was sequenced at Novogene, South Korea, using an Illumina NovaSeq 150 bp paired-end sequencer. The NEBNext® Ultra™ II DNA Library Prep kit was used to create the DNA library, and the Illumina Novaseq PE150 platform was employed for high-throughput sequencing. FASTQC (Version 0.72+galaxy1) and Trimmomatic (Version 0.38.0) were utilized for quality control and the trimming of the reads and adapters, respectively. The Seqtk tool (Galaxy Version 1.3.2) was used to perform subsampling of the reads, and all reads were picked randomly with equal probabilities (9, 10). The Shovill pipeline (Version 3.12.0) was utilized for *de novo* assembly using default parameters, resulting in a single contig that corresponded to the complete phage genome with an average coverage of 16426X (2). To verify the completeness of the assembled contig, Bandage v.0.8.1 was utilized to determine if it could form a circle (11). Using BWA-MEM v.0.7.17.2, the accuracy of the assembly was confirmed by mapping quality-controlled reads back to the assembled genome (9). PhageTerm (version 3.4.0 CPT Galaxy https://cpt.tamu.edu/galaxy-pub) was used to ascertain phage packaging and genome termini. The phage annotation process involved rapid annotation using subsystem technology (RAST) followed by manual confirmation using Blastp (12). tRNAscan-SE 1.21 was used to identify tRNAs (13), while PhagePromoter (Galaxy), Arnold, and phage AI servers were used to predict promoters, rho-independent terminators, and the lifestyle of the phage, respectively (10). VIRIDIC was used to assess the similarity between phage E15 and other phages (Supplementary Figure S1). To classify the species, a genomic similarity threshold of 95% was imposed, whereas a threshold of 70% was applied for classification at the genus level (14). Phylogenetic analysis was performed using the entire genome of the E15 (15). Genomes from *Jersey* phages were obtained from GenBank and compared using the

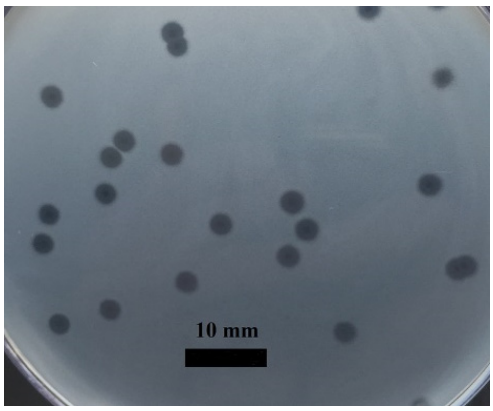

Figure 1: Plaques of the E15 on double-layer agar plates of *S. enteritidis* isolate

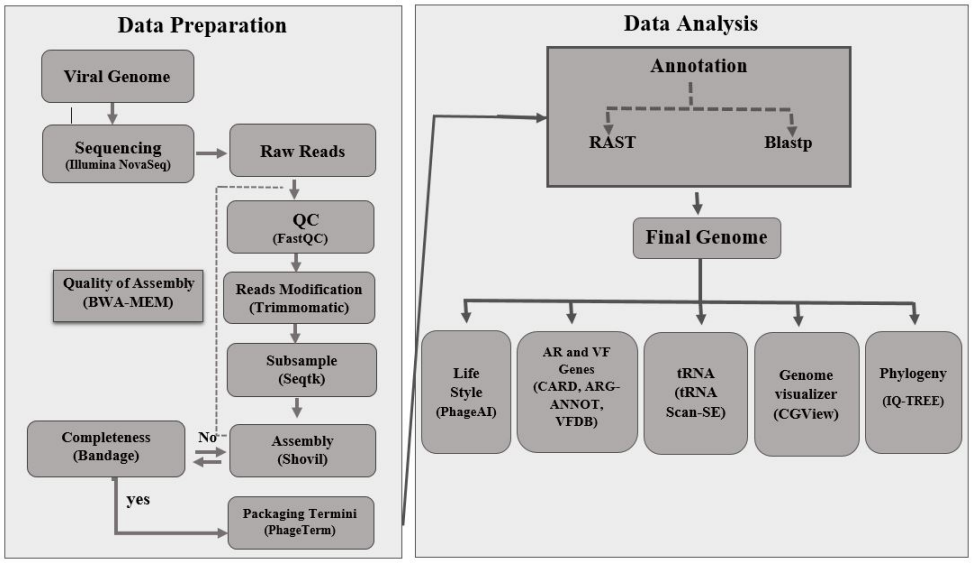

Figure 2: A workflow of the process for preparing and analyzing data to characterize the E15 genome used in this study.

MAFFT online service (16). The alignment files were edited using Mesquite (17), and the tree was generated using IQ-TREE with 1000 bootstrap samples using maximum likelihood methods (18). The CGView Tool was used to construct a map of the phage genome with additional GC skew and GC content analysis (19).

Result and Discussion

Isolation, enumeration and morphology of E15

PPhage E15 was isolated from the Wastewater Treatment Plant of a hospital in Tehran. Transparent and round plaques with slightly turbid halos were observed, ranging in size between 2 and 3 mm in diameter (Fig 1). The phage concentration was determined to be 2×10^{11} PFU/ml.

Genomic analysis

The phage genomic DNA was examined to assess the quality and quantity of the extracted DNA. Through agarose gel electrophoresis, a distinct band with a large molecular size was observed, indicating that the DNA was relatively non-sheared and intact. The workflow of the E15 genome analysis employed in this study is shown in Fig. 2, and the results are provided according to the key steps outlined in this overview.

According to the evaluation of the FastQC tool, the reads were without an adapter, N content, and overrepresented sequences. Also, the reads had a quality score above 35 (20). Upon back-mapping the reads to the genome, it was revealed that over 96.58% of the reads matched the genome, and the median depth reached 8660; consequently, the quality of the assembly was considered acceptable (9). The genome comprises linear double-stranded DNA with a length of 43,048 bp and a GC content of 49.7% (Fig. 3). PhageTerm did not detect distinctive genomic terminals, indicating that the genome might be circularly permutated (21). RAST annotation analysis anticipated a total of 60

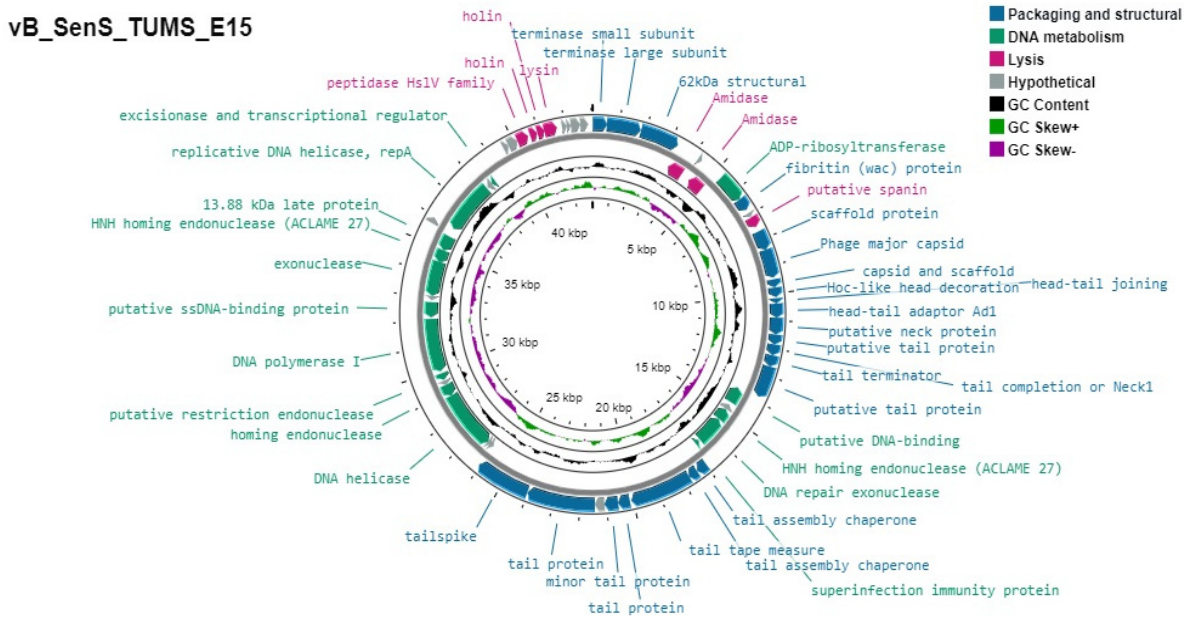

Figure 3: Schematic genomic map of E15 constructed by CGView. CDSs are marked by arrows and colors based on their functions.

coding sequences (CDS), and according to the Blastp, forty-four of the 60 CDSs were annotated as functional genes, including 22 structure-related genes, 7 lysis-related genes, and 15 transcription- and replication-related genes (Supplementary Table S1). More than half of the CDSs associated with phage genomes are still hypothetical and unknown, so functional studies should be performed to determine the function of these unknown elements.

To explore toxins, antibiotic resistance alleles, and virulence factors, the assembled genome was run against the Comprehensive Antibiotic Resistance Database (CARD) (22), ResFinder (23), Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT) (24), the virulence factor database (VFDB) (25), and PlasmidFinder (26) databases using Abricate v.1.0.1 (27). Three promoters from the phage and five from the host that regulate these genes were identified (Supplementary Table S2). The Arnold server identified 37 rho-independent terminators in the genome (Supplementary Table S3), which were assessed according to the presence of a U-rich tail, their position, and predicted stem-loop secondary structure (28). Neither tRNA genes nor genes associated with pathogenicity, toxin production, or lysogeny were predicted. A phylogenetic analysis was conducted to determine the evolutionary background of E15. A maximum likelihood phylogeny was obtained by aligning the whole genome sequences. The results indicated that phage E15 forms a monophyletic group with other phages belonging to the Jerseyvirus genus with high bootstrap support. The results of the phylogenetic assessment are shown in Figtree (Supplementary Figure S2) (29). The phage name was appointed based on Adriaenssens and Brister (30), and the complete genome sequence of Salmonella phage vB_SenS_TUMS_E15 was deposited in the NCBI repository under the accession number ON167532.1.

Conclusion

This study introduces a new *Salmonella* phage, vB_SenS_TUMS_E15, which was obtained from hospital sewage. This is significant because it expands the *Salmonella* library. The E15 genome comprises a 43,048 bp circular double-stranded DNA molecule with a GC content of 49.7%. Bioinformatics analysis indicated that E15 is a new member of the *Jersyvirus* genus in the *Guernseyvirinae* subfamily associated with *Salmonella* phages. It does not contain any lysogenic or toxic genes, which makes it a potential candidate for phage therapy. According to the above characteristics, these phages can be a good and practical target for phage therapy in *Salmonella* infections.

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Conflict of interest

The authors have no conflict of interest.

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/2234368469

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