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# Complete Genome Sequence of *Escherichia coli* - Specific Phage KFS-EC1 Isolated from a Slaughterhouse

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***Escherichia coli*-specific phage, KFS-EC1, was isolated and purified from a slaughterhouse. The complete genome of the phage was obtained using Illumina MiSeq platforms. Its assembled genome consisted of a single chromosome of 164,715 bp with a GC content of 40.5%. The phage genome contained 170 hypothetical and 101 functional ORFs, and exhibited orthologous average nucleotide identity values of >95% with other *E. coli* phages belonging to the family *Straboviridae*. Additionally, phylogenetic analysis revealed that KFS-EC1 was finally classified into the family *Straboviridae* of the genus *Caudoviricetes*. The genome has been deposited in GenBank under the accession number NC_055757.1.**

**Keywords:** **Bacteriophage, *Straboviridae*, *Escherichia coli*, whole genome sequencing**

Bacteriophages (phages) have garnered interest as potent biocontrol agents against *Escherichia coli* due to their ubiquitous existence, specificity, bactericidal effect, and safety [1]. Recent advancements in sequencing and bioinformatics technologies have expanded an understanding of phage diversity and enabled in-depth analysis of novel genes and differences in the genomes among the reported phages [2]. Along with this trend, the International Committee on Taxonomy of Viruses (ICTV) amended and ratified phage taxonomy by transitioning from morphology-based to genome-based classification in 2022. The significant alterations in this update involved the elimination of morphology-driven families, such as *Myoviridae*, *Siphoviridae*, and *Podoviridae*, and the replacement of the *Caudovirales* order to the class *Caudoviricetes* for categorizing all tailed phages possessing icosahedral capsid and a double-stranded DNA genome [3]. These paradigm shifts have necessitated the re-evaluation of previously classified phages. Thus, this study presents a complete genome sequence and revised taxonomic identification of *E. coli*-specific phage KFS-EC1, aligning it with the updated ICTV standards.

KFS-EC1 was isolated and purified from slaughterhouse wastewater in Daegu, Korea [4]. For phage isolation, 1% (v/v) host culture of *E. coli* ATCC 43895 was incubated with 225 ml of tryptic soy broth (TSB, Difco Laboratories, USA) and 25 ml of the sample at 37℃ for 16 h. After centrifugation at 2,400 ×g for 20 min and filtration of the supernatant using a 0.22-μm pore size filter (Advantec MFS Inc., USA), the presence of phage in the filtrate was confirmed via plaque assay. This involved placing a mixture of host and phage suspension on the surface of pre-solidified tryptic soy agar (TSA) plates and incubating them at 37℃ for 16 h. The isolated phage was propagated by incubation with 1% (v/v) host culture in TA broth (8 g/l nutrient broth, 5 g/l NaCl, 0.2 g/l $MgSO_4$, 0.05 g/l $MnSO_4$, and 0.15 g/l $CaCl_2$) at 37℃ for 2 h, followed by adding phage solution and re-incubation at 37℃ for 2 h. After centrifugation of the mixture at 6,000 ×*g* for 20 min, supernatant was filtrated

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using a 0.22-μm filter. This propagation was repeated with increasing volumes of host culture and TA broth. Afterward, phage purification was performed by precipitation using 10% (w/v) polyethylene glycol 6000 (Sigma-Aldrich Co., USA) and CsCl-gradient ultracentrifugation at 39,000 ×*g* for 2 h. Finally, the obtained bluish band was dialyzed using sodium chloride-magnesium sulfate buffer (50 mM/L Tris-HCl, 100 mM NaCl, and 10 mM MgSO4, pH 7.5). The purified phage (KFS-EC1) was stored in a glass vial at 4℃ for further analyses.

The genomic DNA of KFS-EC1 was extracted using a Phage DNA Isolation Kit (Norgen Biotek Co., Canada) according to the manufacturer's instructions. Quantification of the DNA was carried out using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, USA). Subsequently, whole genome sequencing of the phage was performed using the Illumina Miseq platform with paired-end reads of 2 × 150 bp size. Error correction of the generated raw reads was conducted by quality filtering of the Illumina raw reads. The reads that had Phred-score of Q30 and above were collected and assembled using the SPAdes genome assembler (Illumina Inc., USA). The complete genome of KFS-EC1 was annotated using the Rapid Annotations Systems Technology (RAST) server [5] and BLASTP, and the genes linked to lysogenic properties were confirmed using the PHASTER's database (https://phaster.ca/). After categorizing of the annotated open reading frames (ORFs), a genome map of KFS-EC1 was generated using Geneious software (v. 11.1.5, Biomatters Ltd., New Zealand). In addition, the genome of KFS-EC1 was compared with that of similar *E. coli*-specific phages listed in the National Center for Biotechnology Information (NCBI) database, and their average nucleotide identity (ANI) values were determined using OrthoANI Tool (v. 0.93) [6]. Phylogenetic analysis of KFS-EC1 was then performed using the Virus Classification and Tree Building Online Resource [7].

The genetic characteristics of KFS-EC1 are shown in Table 1. The genome of KFS-EC1 consisted of one contig with a genome size of 164,715 bp and a GC content of 40.5%. In addition, its genome was predicted to comprise 170 hypothetical ORFs and 101 functional ORFs, and no tRNA was detected. Although some lytic phages have tRNAs for supporting protein synthesis, but their presence is not universal across all phages. This is because tRNA belongs to the accessory genome that is not an essential component [8]. Furthermore, the functional ORFs were categorized into four groups, including nucleotide metabolism, phage structure and packaging, host lysis, and additional functions (Fig. 1). Expressly, 42 genes related to nucleotide metabolism were confirmed, such as nucleotide replication, DNA repair, and its regulation, which encoded RNA polymerase, DNA polymerase, DNA ligase, DNA helicase, endonuclease, etc. Furthermore, 38 genes involved in the phage structure and packaging were found, such as capsid protein, baseplate and wedge proteins, phage tail, tail fibers, phage neck, phage prohead assembly and capsid scaffolding proteins, and phage tail assembly and scaffolding protein. The main components associated with host lysis (marked as pink arrows, Fig. 1) were also predicted to encode endolysin (9,050 bp–10,849 bp), phage spanin (73,706 bp–74,029 bp), holin (99,392–100,018), and peptidoglycan hydrolase (162,495 bp–162,884 bp). Endolysins degrade the peptidoglycan layer of the cell wall, whereas holins and spanins disrupt the inner membrane and outer membrane of the host, respectively [9], facilitating effective lysis of the host during phages' lytic

**Table 1. Comparison of genetic characteristics between KFS-EC1 and other similar *E. coli*-specific phages.**

| Phage | Genome size (bp) | GC content (%) | tRNA | Predicted ORF | Query coverage (%) | Identity (%) | ANI (%) | Accession number |
|---|---|---|---|---|---|---|---|---|
| KFS-EC1 | 164,715 | 40.5 | ND | 271 | This study | This study | This study | NC_055757.1 |
| vB_Eco_TB34 | 165,220 | 40.5 | ND | 289 | 95.0 | 98.6 | 96.6 | OX_001802.1 |
| JEP8 | 165,295 | 40.5 | ND | 272 | 95.0 | 98.1 | 95.7 | MT_764208.1 |
| W115 | 163,997 | 40.5 | ND | 268 | 94.0 | 98.4 | 96.5 | ON_286974.1 |
| vB_EcoM-pEE20 | 166,422 | 40.4 | ND | 273 | 95.0 | 97.0 | 96.7 | OP_114734.1 |
| vB_EcoM_PHB13 | 165,641 | 40.4 | ND | 274 | 95.0 | 94.5 | 96.3 | MK_573636.1 |

ORF, open reading frame; ANI, average nucleotide identity; ND, not detected.

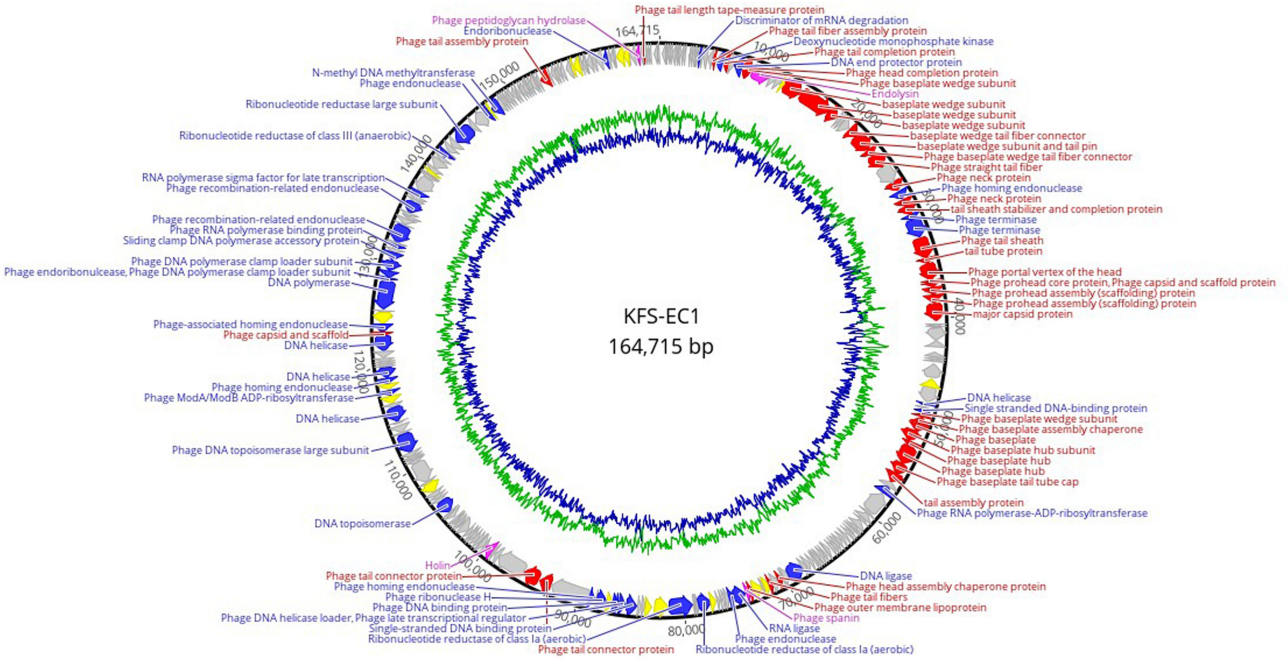


**Fig. 1. Genome map of KFS-EC1.**

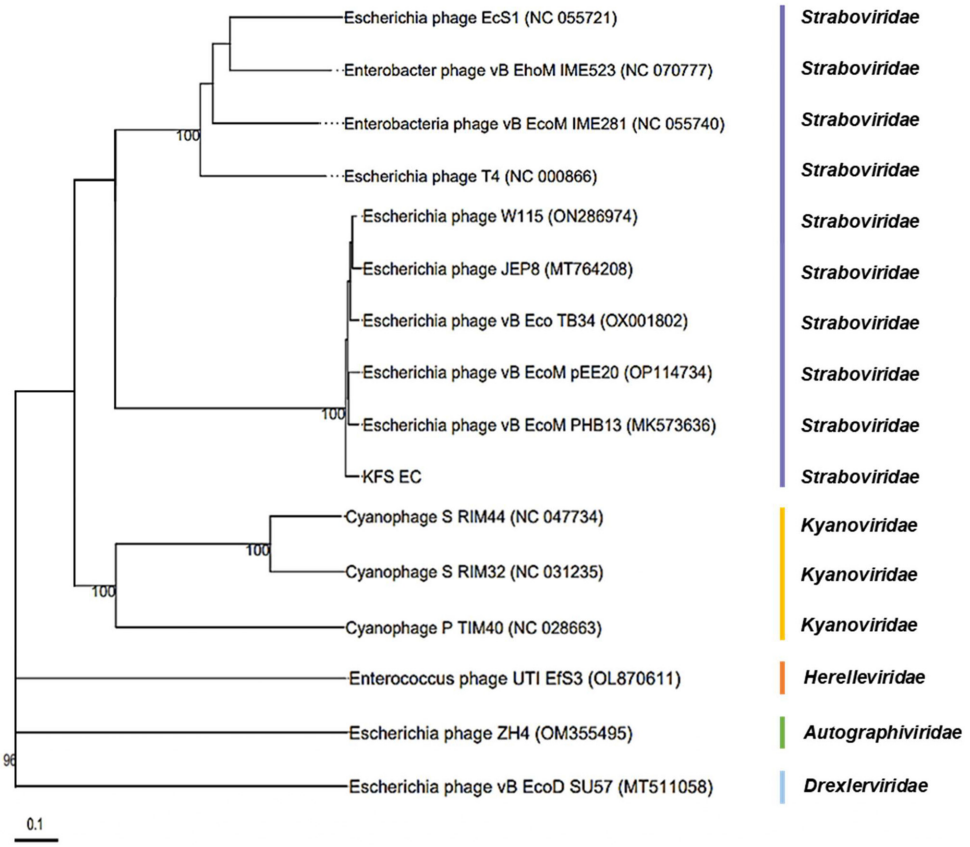


**Fig. 2. Phylogenetic tree between KFS-EC1 and other related taxa within the genus *Caudoviricetes*.**

cycle. In addition, phage-encoded depolymerases, including hydrolases and lyases, can support the control of the host and its biofilm by cleaving the O-glycosidic bonds of bacterial polysaccharides and then degrading polymers [10]. More importantly, KFS-EC1 did not contain genes associated with prophages compared to the PHASTER's database (20 December 2023). Overall, these genetic analyses confirmed that KFS-EC1 can control *E. coli* by expressing lytic action rather than lysogenic activity.

Among *E. coli* phages listed in the NCBI database, the most closely related phages to KFS-EC1 were selected, including vB_Eco_TB34, JEP8, W115, vB_EcoM-pEE20, and vB_EcoM_PHB13, to compare their genetic characteristics. Notably, KFS-EC1 exhibited orthologous ANI values of >95% with all selected phages belonging to the family *Straboviridae* (Table 1). In addition, the phylogenetic analysis of KFS-EC1 (Fig. 2) was performed with ten *Straboviridae* phages, three *Kyanoviridae* phages, one *Herelleviridae* phage, one *Autographiviridae* phage, and one *Drexleviridae* phage to re-classify KFS-EC1 aligning with the updated ICTV standards. The phylogenetic analysis (Fig. 2) exhibited that KFS-EC1 was clustered in the same branch with ten phages within the family *Straboviridae*, indicating a homologous relationship at the nucleotide level. The abolished family *Myoviridae* has recently been amended into two families, *Straboviridae* and *Kyanoviridae*, by significant re-evaluation [3]. The *Straboviridae* family consists of 35 genera, including newly created 11 genera, that exhibit a T4-like morphology possessing an icosahedral head and a contractile tail that terminates in a baseplate with tail fibers. It is consistent with the results of genome annotation that has tail fibers and base plate, as well as the morphology of KFS-EC with contractile tail observed in the previous study [5]. Consequently, our findings finally re-classified KFS-EC1 as a member of the family *Straboviridae*, unlike its previous classification into the family *Myoviridae* [5]. This re-identification significantly can contribute to refining the evolutionary context within the recently revised taxonomy of the genus *Caudoviricetes*.

## Nucleotide Sequence Accession Number

The complete genome of KFS-EC1 has been deposited in the GenBank database under an accession number NC_055757.1.

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## Conflict of Interest

The authors have no financial conflicts of interest to declare.

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