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Complete genome sequence of the bacteriophage vB_EfeM-pEP20 infecting *Escherichia fergusonii*, a new member of the genus *Felixounavirus*§

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*Escherichia fergusonii*를 특이적으로 감염하는 *Felixounavirus*의 새로운 종인 박테리오파지 vB_EfeM-pEP20의 전장 유전체 분석§

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Escherichia fergusonii as an opportunistic pathogen, poses a potential risk to food safety. Although bacteriophages have been considered biocontrol agents against pathogens, phages infecting *E. fergusonii* have rarely been investigated. Thus, we report the complete genome sequence of a newly isolated phage vB_EfeM-pEP20 that infects *E. fergusonii*. The sequenced phage genome was 88,675 bp in length with 38.9% G + C content, encoding 132 ORFs. Genome-based phylogeny revealed that the isolated phage clustered together with the genus *Felixounavirus* and possessed a distinct feature in its DNA packaging system. Therefore, the genetic information will provide essential insights into the biodiversity of *Escherichia* phages.

Keywords: *Escherichia fergusonii*, *Escherichia* phage, *Felixounavirus*, biodiversity

Escherichia fergusonii is a Gram-negative bacterium belonging to the genus *Escherichia* of the family *Enterobacteriaceae* (Farmer *et al*., 1985). It has frequently been isolated from various environmental niches and is now considered one of the most important species in the genus *Escherichia* because of its clinical relevance and ability to cause diseases and infections in humans and animals (Gaastra *et al*., 2014; Tang *et al*., 2022). The prevalence of *E. fergusonii* has been confirmed in various foods and animals, suggesting that this species is potentially widespread throughout the global food chain (Oh *et al*., 2012;

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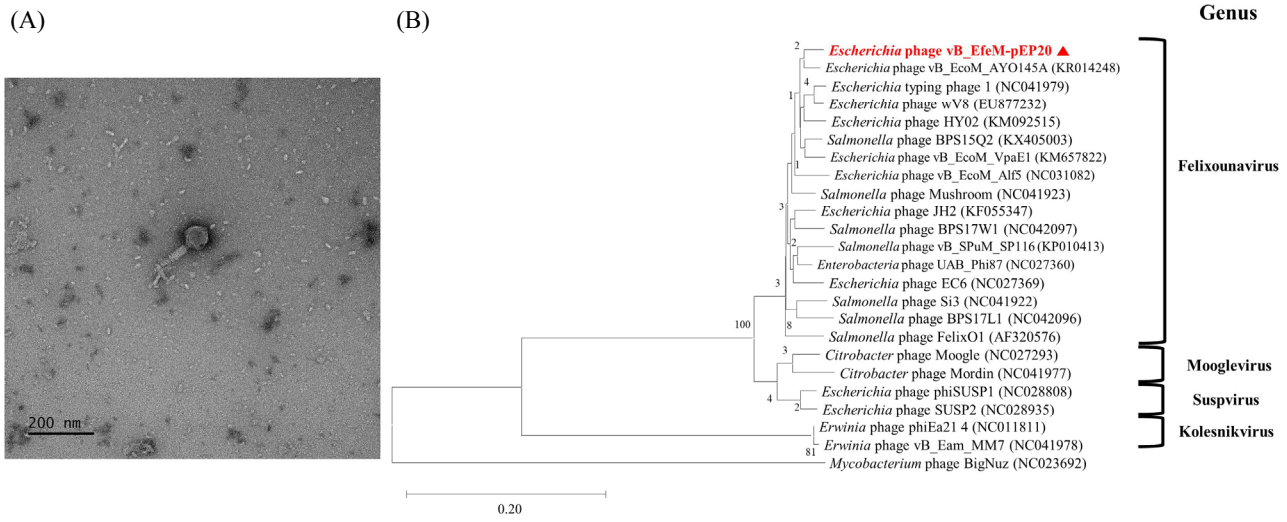

Fig. 1. (A) Transmission electron micrograph of *Escherichia* phage vB_EfeM-pEP20. The scale bar is 200 nm. (B) Genome-BLAST Distance phylogeny (GBDP) tree of 22 species of 4 different genera belonging to subfamily Ounavirinae with the phage vB_EfeM-pEP20. The tree was presented with branch support that was determined using formula D0 and inferred using 100 pseudo-bootstrap replicates with average support of 10% using VICTOR.

Tang *et al*., 2022). Several studies have indicated that *E. fergusonii* obtained from clinical specimens and foods has acquired resistance to various antimicrobial agents and also could likely have multiple antimicrobial resistance (AMR) genes (Simmons *et al*., 2016). Consequently, *E. fergusonii* poses a potential risk to public health and food safety. Bacteriophages (phages) are promising alternatives for controlling bacterial infections in humans and animals. Studies have identified several phages that infect *E. coli*, but studies on phages infecting *E. fergusonii* are very scarce. In this study, we report the complete genome sequence of a novel lytic *Escherichia* phage (isolate designated vB_EfeM-pEP20) infecting *E. fergusonii*.

Escherichia phage vB_EfeM-pEP20 infecting *E. fergusonii* ATCC 35469 was isolated from sewage in Daejeon, South Korea, using a double-layered agar method (Park *et al.*, 2023). The morphological characteristics of the phage were imaged using transmission electron microscopy (TEM) and classified according to the International Committee on Taxonomy of Viruses. Phage genomic DNA was extracted by Macrogen and sequenced on an Illumina platform by preparing a DNA library using the TruSeq Nano DNA library kit (Illumina). Paired-end reads (2,438,804,884 bp; 16,157,714 reads) were assembled *de novo* using SPAdes (v.3.13.0). The putative open reading frames (ORFs) were annotated using the RAST server (https://rast.nmpdr.org/rast.cgi) and BLASTP (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and conserved domains were determined using InterProScan (https://www.ebi.ac.uk/interpro/search/sequence/) searches. The number of tRNAs was determined using tRNAscan-SE v2.0 (http://lowelab.ucsc.edu/tRNAscan-SE/index.html), and the prediction of phage termini and packaging mechanism was predicted using PhageTerm through the Galaxy server (https://cpt.tamu.edu/galaxy-pub). Transmembrane domains and signal peptides were obtained from DeepTMHMM 1.0.19 (https://dtu.biolib.com/DeepTMHMM). Phylogenetic analyses based on the whole genome and *terminase large subunit* were conducted using VICTOR (https://ggdc.dsmz.de/victor.php) and MEGAX (ver. 10.0) (Kumar *et al.*, 2018), respectively.

TEM revealed that vB_EfeM-pEP20 has a non-contractile tail and an icosahedral head, indicating that it belongs to the family *Myoviridae* (Fig. 1A). The phage vB_EfeM-pEP20 had 88,675 bp linear double-stranded DNA (38.9% G + C content) with 593 bp direct terminal repeats (DTRs), consisting of 132 ORFs; only 44 of the ORFs were displayed as functionally putative proteins (Table 1 and Supplementary data Table S1). Direct genome comparison against the GenBank database revealed that the sequenced genome of vB_EfeM-pEP20 was similar to that of members of *Felixounavirus* (felixO1-like phages) and was most similar to that of the *Escherichia* phage vB_EcoM_AYO145A (KR014248; query coverage, 95%;

Table 1. The features of *Escherichia* phage vB_EfeM_pEP20 genome

Features	Value
Contig	1
Genome size (bp)	88,675
G + C %	38.93
Total genes	158
Protein-coding genes (CDS)	132
tRNA	26

identity, 93.7%). A total of 26 tRNAs located between 72,831 and 77,944 in the genome were detected in phage vB_EfeM-pEP20, which was the highest tRNA number among the 16 members of *Felixounavirus* currently available in GenBank (Supplementary data Table S2). To date, several studies have focused on the characteristics of the genes encoding DNA polymerase and tail fiber protein of *Felixounavirus* (felixO1-like phages) (Wang *et al.*, 2015), therefore, we also compared the two genes of the phage vB_EfeM-pEP20. (i) ORF 61 encoding DNA polymerase in the genome of vB_EfeM-pEP20 showed relatively high sequence similarity with vB_EcoM_AYO145A (YP_002922886.1, 97.2%), which might be used as a selection marker for *E. coli trxA* (thioredoxin) gene related to being felixO1-like phages propagate (Šimoliūnienė *et al.*, 2021). (ii) Among the 132 ORFs in vB_Efe-pEP20, ORF 84, which encodes the tail fiber protein, showed the lowest similarity with vB_EcoM_AYO145A (YP_009200817.1, 66%), thus demonstrating the variability of those related to the recognition of bacterial receptors. According to the previous report (Wei *et al.*, 2021), the termini region of *Felixounavirus* can be distinguished from that of other related phages. Therefore, we conducted a *terminase large subunit* gene-based phylogenetic analysis, and the resultant phylogeny revealed that the DNA packaging system of vB_Efe-pEP20 was clustered together with other members of *Felixounavirus* family and clearly separated from those of the other phage genera with six other well-known termini region types (Supplementary data Fig. S1). Moreover, phylogenetic analysis based on the whole genome showed that the phage clustered together with other members of the genus *Felixounavirus* (Fig. 1B). Based on the genomic characteristics and phylogenetic analysis, the newly isolated *Escherichia* phage vB_EfeM_pEP20 could be considered a new member of the genus *Felixounavirus* and its genetic information will provide insights into the genetic diversity of *Escherichia* phages. To the best of our knowledge, this is the first report on the complete genome sequence of phage infecting *E. fergusonii*.

Strain and nucleotide sequence accession number

The complete genome sequence of *Escherichia* phage vB_EfeM-pEP20 has been deposited in DDBJ/END/GenBank under accession number OQ743992 and the phage has been deposited in the Korea Collection for Type Cultures (KCTC) under accession number KCTC 4848.

적 요

*Escherichia fergusonii*는 종 *Escherichia*에 속하는 기회감염 병원체로서 식품안전성을 포함한 공중보건에서의 항생제 내성 출현이 급격하게 증가하고 있다. 박테리오파지는 이러한 병원체를 제어할 수 있는 생물제제로 알려져 있다. 본 연구는 *E. fergusonii*를 특이적으로 감염하는 박테리오파지 vB_EfeM-pEP20의 전장 유전체를 최초 보고한다. 파지 vB_EfeM-pEB20의 전장 유전체의 크기는 88,675 bp이고, G + C 함량은 38.9%이며, 총 132개의 단백질 코딩 유전자를 보유한다. 전장 유전체 기반 계통수 분석 결과, 종 *Felixounavirus*에 속하는 것으로 밝혀졌고, 그 종 중에 tRNA의 수가 가장 많았다. 본 파지의 DNA polymerase와 tail fiber protein을 암호화하는 두 가지의 유전자를 종 *Felixounavirus*에 속하는 파지들과 비교한 결과, 숙주 세균을 감염시키는 본 파지의 자체적 유전체 특성을 밝혔고, 아과 *Ounavirinae*에 속하는 다른 파지들과 명백하게 구분되는 DNA packaging system을 갖고 있는 것으로 확인되었다. 따라서, 본 연구는 *E. fergusonii*를 감염시키는 파지 vB_EfeM-pEP20의 전장 유전체 분석의 최초 보고를 통해 종 *Felixounavirus*의 생물다양성에 중요한 통찰력을 제공한다.

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Conflict of Interest

All authors disclose no conflicts of interest relevant to this research.

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