Complete genome analysis of enterotoxigenic *Escherichia coli* K88 phage vB_EcoP_E212, a novel genus *Lederbergvirus*

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Research Article

Keywords: enterotoxigenic *Escherichia coli* K88, phage, genome analysis, biological characteristics

Posted Date: September 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-852746/v1>

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Abstract

The features and genome annotation of a newly bacteriophage v B_EcoP_E212 (referred to as E212) which isolated from farm sewage collected in Jilin, China was describes in this study. Bacteriophage E212 belongs to the family *Podoviridae*, order *Caudovirales* through transmission electron microscopy. This phage specifically infects enterotoxigenic *Escherichia coli* K88. The dsDNA molecule of phage E212 was 38252 bp in length and contained 46.98% G + C content. It has been predicted to contain 53 ORFs, and no tRNAs. Phage E212 carried the integrase gene, and no homologues of virulence factors or antimicrobial resistance genes were found in this phage. Phage E212 was assigned to the genus *Lederbergvirus* in accordance with nucleotide sequence alignment and phylogenetic analysis.

Introduction

Diarrheal disease due to enterotoxigenic *Escherichia coli* (ETEC) is the most common enteric colibacillosis encountered in neonatal and early-weaned piglets [1]. The main virulence attributes of ETEC are adhesins (fimbriae) and enterotoxins (proteins) [2], both essential for disease development. Among the different ETEC, including K88 (F4), K99 (F5), 987p (F6), F41 and F18, the K88 or F18 are the most prevalent form of *E. coli* infections [3]. *E. coli* colonizes the small intestine via fimbrial adhesins that bind to glycoprotein receptors on the enterocyte brush border and subsequently secretes heat-labile enterotoxins and/or heat-stable enterotoxins that cause diarrhea by altering tight junction integrity and disrupting paracellular passages for ions, solutes and water [4]. Treatment of ETEC infection could be common by the administration of antibiotics, but antibiotic abuse may lead to drug residues [5], immunity decline in animals [6], bacterial resistance [7] and other adverse reactions. Increasing bacterial resistance poses a major treat to human, animal health, and environment worldwide. Consequently, there is an urgent need to develop new strategy against bacterial pathogens is essential. Bacteriophage (phage) is increasingly considered as a valuable alternative to antibiotics [8]. Bacteriophages are viruses that infect bacterial cells. As one of the most abundant organisms on earth, bacteriophages play important roles in maintaining the natural abundance and distribution of microorganisms [9]. Compared to traditional antimicrobial therapy, there are many major advantages of phage therapy: high bactericidal activity, low inherent toxicity, low cost, narrow potential for including resistance. In this study, we have sequenced and analyzed the complete genome of a newly isolated ETEC K88 phage.

Materials And Methods

Bacterial strains and Phage isolation

The bacterial strains used in this study are shown in **Table 1**. Enterotoxigenic *Escherichia coli* K88 (CVCC 83902) used as a host was obtained from China Veterinary Culture Collection Center (CVCC). All strains were cultured on LB with constant shaking at 37°C or were stocked in LB containing 50% glycerol and stored at -80°C. Isolation and purification of phage vB_EcoP_E212 (referred to as E212) from farm sewage in Jilin province was done according to procedures described previously by Zhang et al[10].

Host range investigation and efficiency of plating analysis

The lytic capacity of phage E212 was determined by the spot test method in accordance with the ability to form lysis zone on lawn cultures of different strains [11]. Phage E212 was purified ($10\mu\text{l}$, 10^9 PFU/mL) and directly spotted on the surface of the bacterial lawn culture plates, and incubated overnight at 37°C . Checked for appearance of the clear zones around the phage droplet on the plates. Efficiency of plating (EOP) was used to evaluate the ability of phage lysing bacterial strains (positive spot test). The phage titer on the host strain was calculated as $\text{EOP}=1$, and other EOPs were obtained by dividing the phage titer on the other test strains by the phage titer on the host strain.

Transmission electron microscope

Purified phage suspension (10^9 PFU/mL) was absorbed onto carbon coated copper grids for 10 min, and then negatively stained with 2% (w/v) uranyl acetate, followed by examination using a JEM-2100EX transmission electron microscope (TEM) (JEOL CO., Tokyo, Japan) [12].

Phage DNA purification and sequencing

Phage genomic DNA was extracted from a preparation with a high titer of phage particles (10^{10} PFU/mL) using the phenol-chloroform-isoamyl alcohol method as described by Sambrook et al [13]. The DNA library was established in accordance with the protocol of the Illumina TruSeqTM Nano DNA Sample Prep Kit. The whole genome was sequenced using the Illumina NovaSeq sequencing platform (150 bp $\times$ 2) with paired-end reads by Shanghai Personal biotechnology Co., Ltd (China) [14]. A total of 382 Mb of sequence data were obtained with an average reading length of 150 bp. Low quality (Q-value <20 , 98.51%) reads were filtered out through Trimmomatic v 0.36, and approximately 98722 $\times$ depth of coverage among the 25,525,764 reads. A5-MiSeq v20160825 (<https://arxiv.org/abs/1401.5130>) and SPAdes v3.12.0 (<http://cab.spbu.ru/files/release3.12.0/manual.html>) were used to assemble high quality sequencing data to constrict contig and scaffold. MUMmer v3.1 (<http://mummer.sourceforge.net/>) and Pilon v1.18 (<https://github.com/broadinstitute/pilon>) were subsequently applied to fill the remaining local inner gaps and correct single-base polymorphism for the final assembly results.

Genome analysis

Open reading frames (ORFs) were identified using the GeneMark Server (<http://topaz.gatech.edu/GeneMark/genemarks.cgi>) and the RAST server (<http://rast.nmpdr.org/rast.cgi>). The final assembled genome sequence was used to search the current protein and nucleotide databases (<http://www.ncbi.nlm.nih.gov/>) using the Basic Local Alignment Search Tool (BLAST). BLASTp (<https://blast.ncbi.nlm.nih.gov/Blast>) was used to identify the putative functions of the encoded proteins, and BLASTn (<https://blast.ncbi.nlm.nih.gov/Blast>) was used to compare phage genome sequence similarity. Putative tRNA sequences were identified using tRNAscan-SE [15]. The ResFinder sever [16] (<https://cge.cbs.dtu.dk/services/ResFinder/>) and Virulence Factor

Predictor [17] (<https://cge.cbs.dtu.dk/services/VirulenceFinder/>) were used to identify antimicrobial resistance determinates in the E212 genome.

The whole genome sequence of phage E212 was performed phylogenetic analysis with 18 published sequences (these phages belong to family *Podoviridae*, genus *Lederbergvirus*) in GeneBank by MEGA-7. The sequence aligned by ClustalW, then, the maximum likelihood method was adopted to draw the phylogenetic tree. The large subunit terminase sequence was employed to determine the DNA packaging strategies of the phage. The large subunit terminase amino acid sequence obtained in this study and those of other phages were selected for multiple alignments using the Clustal W algorithm, and phylogenetic tree was constructed using MEGA7 with the neighbor-joining method. The comparative analyses of phage complete genome sequences, between E212 and the other members of the genus *Lederbergvirus*, were conducted using Easyfig.

Results And Discussion

One phage against Enterotoxigenic *E. coli* K88 (CVCC 83902) was isolated from sewage. We named this phage vB_EcoP_E212 (referred to as E212). TEM analysis revealed that E212 has an icosahedral head ($50 \pm 3\text{nm}$) connected to a tail ($8 \pm 2\text{nm}$). Based on these structural features, E212 was designated as a member of *Podoviridae* family, *Caudovirales* order. (**Fig.S1**). Spot test and EOP results indicated that only host bacteria (CVCC 83902) was lysed by the phage E212 (**Table 1**).

The results of the present study showed that phage E212 has a double-stranded DNA genome with a length of 38,252 bp and an overall G + C content of 46.98%. Using the RAST server, we identified 53 ORFs and predicted 44 putative protein-coding genes in the genome, 31 of which were functionally assigned. Based on bioinformatic predictions, these ORFs were categorized into four functional modules, including phage structure, host lysis, phage DNA packaging and replication and hypothetical protein (**Table S2**). Using tRNAscan-SE, no tRNA genes were predicted. Most of the ORFs of E212 were predicted to start with an AUG codon (47 ORFs, 88.7%), four with GUG codon (7.5%) and two with UUG (3.8%). The three stop codons were present in different proportions, with UGA being the most common (31 ORFs, 58.5%), followed by UAA (18 ORFs, 34%) and UAG (4 ORFs, 7.5%).

To investigate the evolutionary relationship that phage E212 within the genus *Lederbergvirus*, phylogenetic analyses were performed on the whole genome of 18 published phage genome and E212 (Fig. 2a). In phylogram tree the phage E212 was more closely related to *Escherichia* phage phiv205-1, *Escherichia* phage JEP4 and Enterobacteria phage Sf101. The DNA packaging strategies of tailed dsDNA phages can be classified into 6 types (17 subtypes): (a) cohesive ends (5' cos, lambda P2; 3' cos, HK97); (b) headful packaging (P2, P22, Sf6, T4, 933 W, phiPLPE, phiKZ); (c) host ends (Mu, D3112); (d) short direct terminal repeats (DTRs) (T7, N4, C-st); (e) long DTRs (SPO1); and (f) covalently bound terminal proteins (*Bacillus subtilis* phage ϕ 29) [18, 19]. As shown in Fig. 2b, the phage E212 belongs to headful packaging strategy (P22). No homologs of virulence factors (virulence genes for *E. coli*) or antimicrobial resistance genes were found in the E212 genome. However, all *Lederbergvirus* phages including E212 carry an integrase

gene. Therefore, E212 is not suitable for therapy. Phages are being considered valuable alternative antimicrobial solutions, but not all phages can be considered for therapy. For safety reasons, phage genome sequence cannot carry antibiotic resistance, virulence and lysogenic genes.

A total of twenty conserved proteins are homologous (identity > 71%) between E212 and Enterobacteria phage Sf101, among phages within the genus *Lederbergvirus* (Fig. 3). These include the portal, scaffold, capsid, DNA stabilization protein, head assembly protein, injection protein, tail spike, integrase, DNA methylase, DNA binding protein, i-spanin, O protein, holin and lysis.

Conclusion

We therefore conclude that phage E212 is a newly isolated genus *Lederbergvirus*.

Declarations

Nucleotide sequence accession number

The GenBank accession number for phage vB_EcoP_E212 is MZ043897.

Funding

This work was financially supported by the Agricultural Science and Technology Innovation Program of Jilin Province (CXGC2017JQ003).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

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Tables

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Figures

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Figure 1

Figure 2

a: The phylogenetic tree of 19 whole genome of phages within genus Lederbergvirus b: Neighbor-joining phylogenetic tree based on amino acid sequence of terminase large subunit, showing the relationship between phage E212 and other phages. Values at the nodes indicate the bootstrap support calculated from 1000 replicates.

Figure 3

Comparison of the genome of phage E212 to other Lederbergvirus members (Escherichia phage JEP4, Enterobacteria phage Sf101, Enterobacteria phage P22, Enterobacteria phage Sf6) using Easyfig software. The different color arrows represent CDS in the whole genome sequence. The direction of arrow indicated the transcription direction of each CDS. The grey bars indicated the similarity of two pairs of sequences, and the intensity of grey indicated the degree of sequence similarity.

Supplementary Files

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