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ANNOTATED SEQUENCE RECORD

The complete genome sequence of PE3-1, a novel *E. coli* O153 phage

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Abstract: A novel virulent phage PE3-1 against *E. coli* O153 was isolated from an aeration tank in a wastewater treatment plant. Transmission electron microscopy images showed that phage PE3-1 had an icosahedral head and a short tail, which revealed that it was a member of the family *Podoviridae* of the order *Caudovirales*. The complete PE3-1 genome consisted of 39,093 bp and was a linear double-stranded DNA with an average GC content of 49.93 %. Phage PE3-1 showed homology to the T7-like phages in 48 open reading frames (ORFs), but it differed from previously reported *E .coli* phages in morphology and bioinformatics analysis. This indicated that phage PE3-1 is a new member of the genus T7 virus.

Introduction

Waterborne diseases were estimated to be responsible for 4.0 % of all deaths and 5.7 % of the total disease burden worldwide [1]. The conventional strategy for pathogen control is antibiotic therapy. In the long run, in order to avoid the selection of antibiotic-resistant strains, alternative strategies to treat pathogenic bacteria are urgently needed. Phages have high specificity for their bacterial host. Lytic phages can enter their hosts, complete their proliferation, and kill the hosts without harming humans or animals. It is expected that phages can be used as biocontrol agents against bacterial pathogens [2], which is a potentially promising treatment and prevention measure [3–6].

More recently, bacteriophages have been intensively studied with regard to their potential application for the control of *E. coli* in livestock, aquaculture and food products [7–11], but there have been few studies in wastewater treatment systems.

In this work, a novel phage, named PE3-1, against *E. coli* O153 was isolated from an aeration tank in a wastewater treatment plant in Beijing, China. Its morphology, a complete genome sequence were determined, and a preliminary analysis of its genome structure was carried out. This could provide a foundation for the development of biological agents to control with pathogenic bacteria.

Materials and methods

Phage isolation

E. coli O153, the host strain for phage PE3-1, was used to enrich and isolate virulent phages from aeration tank samples in a wastewater treatment plant in Jan. 2013. Isolation, propagation and titration of phages were carried out as described previously [12]. Bacterial nucleic acids were removed from phage lysates by treatment with DNase I (Sigma-Aldrich, Oakville, ON, Canada) and RNaseA (Sigma-Aldrich), and the phage lysates were concentrated using polyethylene glycol 8000 [13]. The phage was then stored in SM buffer (100 mM NaCl, 8 mM $MgSO_{4,5}$0 mM Tris-HCl [pH 7.5]) at 4 °C for further study.

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Phage morphology

The morphology of phage PE3-1 was observed by transmission electron microscopy. Phage particles (approximately 10^{11} PFU/ml) were stained with 2 % (w/v) aqueous uranyl acetate (pH 4.5-5.5) on a carbon-coated grid and then were observed by transmission electron microscopy using a JEM-1400 apparatus at an accelerating voltage of 80 kV. Phage PE3-1 identification was based on its morphological features according to the Eighth Report of the International Committee on Taxonomy of Viruses (ICTV) [14].

DNA sequencing and analysis

Phage DNA was extracted using the SDS-proteinase K method described by Sambrook and Russell [13]. DNA was sequenced using the Illumina Miseq (PE250) platform at Shanghai Majorbio Bio-Pharm Technology Corporation. More than 2,400-fold coverage of the phage genome was achieved by sequencing the cloned fragments. The raw Miseq sequences were trimmed and corrected. The paired-end reads were assembled using SOAPdenovo (http://soap.genomics.org.cn/, v1.05) and GapCloser (v1.04). The base composition of phage PE 3-1 was obtained by sequence assembly using the EditSeq sequence analysis software (DNASTAR). Potential ORFs were predicted using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), and ORFs were further identified using GLIMMER (ver.3.02) [15] and the "Gene Finding in Viral Genomes" function in Softberry (http://linux1.softberry.com/all.htm). Possible tRNAs in the genome were identified using tRNAscan-SE (http://lowelab.ucsc.edu//tRNAscan-SE/). Comparisons of nucleotide acid and predicted protein sequences with other known sequences were performed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Blastp and HHPred (http://toolkit.tuebingen.mpg.de/hhpred) were used together for putative protein function identification. The map of GC content and GCskew was generated using CGView server (http://stothard.afns.ualberta.ca/cgview_server/). The complete genome sequence of phage PE3-1 had been deposited in GeneBank with the accession number "KJ748011".

Results and discussion

Morphology

Transmission electron microscopy images showed that phage PE3-1 has an icosahedral head approximately 50 ± 2 nm in diameter and a short tail about 10 ± 2 nm in length. According to the criteria established in the 8th report of the ICTV [14], it was assigned to the family *Podoviridae* of the order *Caudovirales.*

General features of the phage PE3-1 genome

The complete genome sequence of phage PE3-1 consists of 39,093 bp and is a linear double-stranded DNA with an average GC content of 49.93 % (Fig. 1). There were no tRNAs detected in the genome of phage PE-3, indicating that phage PE3-1 is completely reliant on the host tRNA after entering the host.

The predicted protein sequences of each ORF were compared with those from the NCBI database and HHpred database (Table 1 in the Supplementary Material). In the NCBI database, potential functions of 28 proteins were predicted by functional homology (including 27 genes identified in other phage genomes and one gene identified only in a bacterial genome). A total of 20 putative proteins were hypothetical with unknown functions. The presence of lysis related genes (ORF20, ORF43 and ORF46) and the absence of lysogeny-related genes indicate that phage PE-3 is a lytic phage. In the HHpred database, potential functions of 23 proteins were predicted, and most of them were consistent with the NCBI database. The predicted function of ORF17 was quite different between the two databases: the encoded protein was predicted to be an HNH endonuclease in the NCBI database, but it was a TC3 transposase in the HHpred database. Therefore, the following

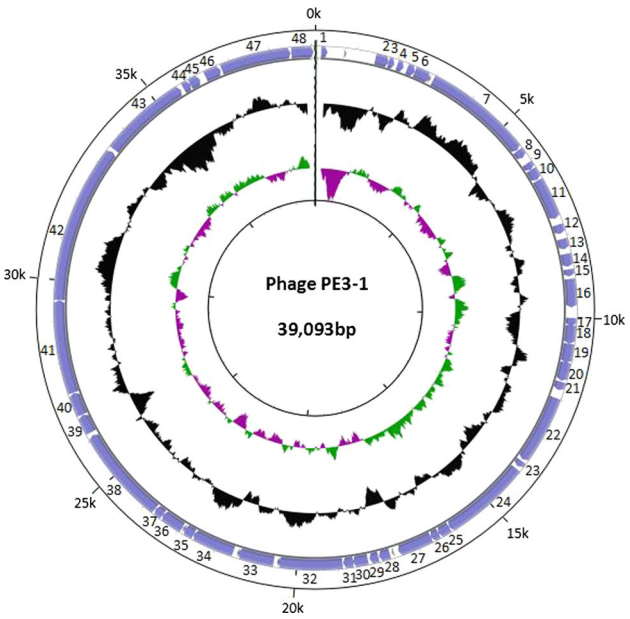

Fig. 1 A circular representation of the phage PE3-1 genome, created using the CGView server. Circles display (1) the physical map scaled in kb, (2) ORFs transcribed in the clockwise direction, (3) GC content, (4) GC skew. Values greater than zero are in green, and those less than zero are in magenta

discussion was based on the analysis results of the NCBI database.

Replication- and structure-related genes

Phage PE3-1 possesses replication-related genes encoding DNA ligase (ORF11), endonuclease (ORF18 and ORF27), helicase (ORF22) and DNA polymerase (ORF24). ORF11 is homologous to the DNA ligase of *Enterobacteria* phage K1F (95 % similarity). ORF18 and ORF27 have 84 % sequence identity to the endonuclease of *Citrobacter* phage CR44b. ORF22 showed 97 % identity to the primase/helicase of *Enterobacteria* phage K1F. ORF24 was similar to DNA polymerase of enterobacteria phage K1F (97 % identity). Phage PE3-1 was predicted to use an excision repair pathway based on the encoded enzymes (DNA helicase, endonuclease, polymerase and ligase). DNA packaging protein (ORF45) and DNA maturation protein (ORF47) are proteins involved in completing the packaging process. A gene encoding terminase, which plays an essential role in the double-stranded DNA packaging process, was not found in the phage PE3-1 genome. Terminase, which is generally composed of two subunits, identifies the pre-capsid protein and the specific packaging sites, providing energy for the packaging process through hydrolysis of ATP [16, 17]. The lack of a terminase gene suggests that phage PE3-1 might depend on the terminase of the host for this function.

Phage PE3-1 is dependent on RNA from its host for gene transcription and translation, but it also possesses its own RNA-related enzymes such as RNA polymerase (ORF7) and RNA-binding protein (ORF37) to regulate gene expression. ORF7 is homologous to RNA polymerase of *Enterobacteria* phage K1F with 99 % sequence identity. ORF37 had 61 % sequence identity to an RNA-binding protein of *Gamma proteobacterium* WG36.

Ten genes related to the structure of phage PE3-1 were identified in its genome, including phage virion protein (ORF31, ORF33 and ORF39-42), tail tube (ORF36 and ORF38) and head-to-tail connector (ORF32). The tail contains a nanomotor that translocates the dsDNA chromosome into the virion during the assembly of progeny phage particles in the lytic growth cycle [18]. This function is essential for successful assembly. The tail proteins are capable of binding to the host receptor and determine the host range of the phage, which is of interest for phage therapy [19].

HNH endonuclease

HNH endonuclease can bind DNA, which was an important component of the R-M (restriction-modification) system for bacterial resistance to phage infection and the insertion of foreign DNA [20]. HNH endonucleases have been identified in a number of phages and have various roles in the phage life cycle [21]. Using blastp at NCBI, ORF48 and ORF17 in the phage PE3-1 genome were found to be homologous to a putative HNH endonuclease of *E. coli* (AA identity = 47 %) and *Kluyvera* phage Kvp1 (AA identity = 51 %), respectively. This suggests that horizontal gene transfer has occurred between phage PE3-1 and *E. coli* for a long time, and this is likely to be related to the resistance of *E. coli* to phage and to be beneficial for maintaining the balance between species. If the HNH endonuclease gene is missing, it might allow the phage to eliminate *E. coli* more efficiently, but the phage might regain this gene by gene transfer in a short time.

Host RNAP inhibitor

ORF48 in phage PE3-1 showed 47 % sequence identity to a host RNAP inhibitor found in members of the family *Enterobacteriaceae*. Phages produce a host RNAP inhibitor to interfere with normal genetic function and restrain proliferation of the host bacterium. The host RNAP inhibitor is usually recognized as a pathogenic factor for the host. In addition, it has been reported that some phages might produce methyltransferase or methylase to restrain expression of DNA of the host bacteria or modify their own DNA to escape hostile attacks from the host bacterium [22, 23]. The effects of these inhibitors are limited because bacteria have defense mechanisms, for example, an R-M system, CRISPR-Cas systems, and abortive-infection mechanisms [20, 23, 24]. However, the evolutionary relationship between the phage and the host bacterium and the mechanism of resistance are not fully understood.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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

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