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GENOME SEQUENCES

Complete Genome Sequence of *Klebsiella pneumoniae* Siphophage Seifer

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ABSTRACT Carbapenemase-producing *Klebsiella pneumoniae* poses a significant public health threat due to its resistance to antibiotics. Siphophage Seifer was isolated and characterized as part of an effort to develop phage therapeutics to control this pathogen. This report describes the complete genome sequence of phage Seifer, which is a distant member of the χ-like siphovirus phage cluster.

Klebsiella pneumoniae is an opportunistic human pathogen often linked to hospital-acquired infections (1). *K. pneumoniae* strains that carry the plasmid-borne and highly mobile *K. pneumoniae* carbapenemases (bla_{KPC}) are of special concern due to their ability to degrade carbapenem antibiotics (1–3). Phages targeting KPC-positive *K. pneumoniae* could be used as alternatives to antibiotic treatments.

Phage Seifer was isolated from a wastewater sample collected from Brazos County, TX, in 2015 using a KPC-positive *K. pneumoniae* clinical isolate of sequence type 258 as the host. Host bacteria were cultured on tryptic soy broth or agar (Difco) at 37°C with aeration. Phages were cultured and propagated using the soft-agar overlay method (4). The phage was identified as a siphophage using negative-stain transmission electron microscopy performed at the Texas A&M University Microscopy and Imaging Center as described previously (5). Phage genomic DNA was prepared using a modified Promega Wizard DNA cleanup kit protocol as described previously (5). Pooled indexed DNA libraries were prepared using the Illumina TruSeq Nano LT kit, and the sequence was obtained from the Illumina MiSeq platform using the MiSeq V2 500-cycle reagent kit following the manufacturer's instructions, producing 697,877 paired-end 250-bp reads for the index containing the phage Seifer genome. FastQC 0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to quality control the reads. The reads were trimmed with FastX Toolkit 0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/download.html) before being assembled using SPAdes 3.5.0 (6). Contig completion was confirmed with PCR using primers (5′-TGTATCCTACGCTCGTTCCC-3′, 5′-CCGATTATGACGCCGCTATG-3′) facing off the ends of the assembled contig and Sanger sequencing of the resulting product, with the contig sequence manually corrected to match the resulting Sanger sequencing read. GLIMMER 3.0 (7) and MetaGeneAnnotator 1.0 (8) were used to predict protein coding genes with manual verification, and tRNA genes were predicted with ARAGORN 2.36 (9). Rho-independent terminators were identified via TransTerm (http://transterm.cbcb.umd.edu/). Sequence similarity searches were performed using BLASTp 2.2.28 (10) with a maximum expectation cutoff of 0.001 against the NCBI nonredundant (nr), UniProt Swiss-Prot (11), and TrEMBL databases. InterProScan 5.15-54.0 (12), LipoP (13), and TMHMM 2.0 (14) were used to predict protein function. All analyses were conducted at default settings via the CPT Galaxy (15) and WebApollo (16) interfaces (https://cpt.tamu.edu/galaxy-pub).

Phage Seifer has a complete genome of 58,197 bp assembled at 212-fold coverage. It has a GC content of 56% and a coding density of 95%. Seifer is a distant member of the χ-like phage cluster (17), with similarity to phage χ in the left arm of the genome.

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Out of 82 total predicted proteins in phage Seifer, 43 and 45 proteins show similarity to proteins encoded by *Salmonella* phage χ (GenBank accession no. NC_025442) (18, 19) and *Escherichia* phage Utah (GenBank accession no. KY014601) (20), respectively, based on BLASTp with an E value of <0.001. The 12-bp 5′ extended overhang sequences (5′-GGTGCGCAGAGC-3′) in phages χ and Utah were identified in phage Seifer at the beginning of the genome. As determined by BLASTn against the NCBI nucleotide (nt) database, Seifer is closely related to *Klebsiella* phage Soft (GenBank accession no. MN106244) at the nucleotide level by sharing 85% overall identity (E value, 0).

Data availability. The genome sequence of phage Seifer was submitted to GenBank under the accession no. MH817999. The associated BioProject, SRA, and BioSample numbers are PRJNA222858, SRR8556780, and SAMN10904482, respectively.

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