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GENOME SEQUENCES

Complete Genome Sequence of *Klebsiella pneumoniae* Podophage Pylas

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ABSTRACT Carbapenemase-producing *Klebsiella pneumoniae* is an important opportunistic pathogen due to its drug resistance. This study reports on the isolation and characterization of a podophage, named Pylas, infecting this bacterium. The complete genome of phage Pylas is described, and it is distantly related to the well-studied phage N4.

Multidrug-resistant *Klebsiella pneumoniae* poses an urgent threat to public health due to its ability to infect patients with a compromised immune system (1, 2). *K. pneumoniae* strains producing carbapenemases are resistant to a broad range of antibiotics and can cause infections leading to high mortality rates (3). Phages infecting *K. pneumoniae* may be used in new therapies for treating this pathogen.

Phage Pylas was isolated from wastewater collected in College Station, TX, in 2015 against a carbapenemase-producing *K. pneumoniae* isolate. 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 podophage 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 (5). Pooled indexed DNA libraries were prepared using the Illumina TruSeq Nano LT kit, and the sequence was obtained with the Illumina MiSeq platform using the MiSeq V2 500-cycle reagent kit following the manufacturer's instructions, producing 773,101 paired-end 250-bp reads for the index containing the phage Pylas 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′-TTGAGTCTGTT CACGCCAAC-3′, 5′-TACCAACAGTTGACCCAGCA-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 termination sites were identified via TransTermHP (http://transterm.cbcb.umd.edu/). Sequence similarity searches were done 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 Pylas was assembled at 79.6-fold coverage into a unit genome of 70,408 bp (17). The GC content of Pylas is 41%, in contrast to the 57% GC content of its *Klebsiella* host (18). As determined by BLASTp, Pylas shares 30 proteins with *Escherichia coli*

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podophage N4 (GenBank accession no. NC_008720) (E value, $<10^{-3}$) (19). These shared proteins are involved in DNA replication, transcription, DNA packaging, and morphogenesis. Similar to N4, the Pylas genome has direct terminal repeats, which were predicted by PhageTerm (17) to be 769 bp long; the Pylas genome is generally syntenic with N4. Pylas is closely related to *Klebsiella* phage KpCHEMY26 (GenBank accession no. MN163281), sharing 94% overall nucleotide identity (E value, 0) as determined by BLASTn against the NCBI nucleotide (nt) database. The predicted lysis cassette of Pylas is composed of a holin-antiholin pair, an embedded inner-outer spanin pair, and a peptidoglycan hydrolase endolysin.

Data availability. The genome sequence of phage Pylas was submitted to GenBank under the accession no. MH899585. The associated BioProject, SRA, and BioSample accession numbers are PRJNA222858, SRR8556430, and SAMN10909361, respectively.

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