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GENOME SEQUENCES

Complete Genome Sequence of *Stenotrophomonas maltophilia* Siphophage Silvanus

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ABSTRACT *Stenotrophomonas maltophilia* is an opportunistic Gram-negative bacterium capable of causing respiratory infections. *S. maltophilia* siphophage Silvanus was isolated, and its 45,678-bp genome is not closely related to known phages based on whole-genome comparative genomics analysis. It is predicted to use *cos*-type packaging due to the similarity of its large terminase subunit to that of phage HK97.

Stenotrophomonas maltophilia is an emerging Gram-negative, multidrug-resistant pathogen most associated with respiratory infections in humans (1). With a goal of using phage as potential control for this pathogen, we report here the isolation and genome annotation of Silvanus, a siphophage targeting *S. maltophilia*.

Phage Silvanus was isolated from a soil sample collected from a horse pasture in College Station, TX (GPS coordinates 30°33′04.4″N, 96°18′44.0″W), in January 2019. Silvanus was isolated and propagated with the soft-agar overlay methods described previously (2) using an *S. maltophilia* strain (ATCC 51331) grown aerobically at 30°C in nutrient broth or agar (BD). Samples were negatively stained with 2% (wt/vol) uranyl acetate and imaged by transmission electron microscopy (TEM) at the Texas A&M Microscopy and Imaging Center (3). DNA was purified using a Promega Wizard DNA cleanup system as described (4), and the libraries were prepared using a Swift 2S Turbo library preparation kit and sequenced on an Illumina MiSeq machine with paired-end 150-bp reads and V2 300-cycle chemistry. The sequence reads were quality controlled with FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc) and trimmed with FASTX-Toolkit v0.11.6 (http://hannonlab.cshl.edu/fastx_toolkit/). Genomes were assembled from 85,453 trimmed reads with SPAdes v3.5.0 (5), and a contig with 138-fold sequencing coverage was obtained. The genome was closed by PCR and Sanger sequencing using forward primer 5′-CATCGTGTGTGGGCGAAATC-3′ and reverse primer 5′-TGAACCCCTGAGTTTCGTGG-3′. PhageTerm was used to predict phage termini from raw sequencing reads (6). The genome was assembled and annotated with the CPT Galaxy-Apollo phage annotation platform (https://cpt.tamu.edu/galaxy-pub) (7–9). Gene calling was conducted with GLIMMER v3 and MetaGeneAnnotator v1.0 (10, 11). tRNAs were detected with ARAGORN v2.36 and tRNAscan-SE v2.0 (12, 13). Gene function predictions were determined using InterProScan v5.48 (14) and BLAST v2.9.0 (15) against the NCBI nonredundant (nr) and Swiss-Prot databases (16), TMHMM v2.0 (17), HHPred, LipoP v1.0, and SignalP v5.0 (18–20). The genome-wide DNA sequence similarity to the top BLAST nucleotide hits was calculated with progressiveMauve v2.4 (21). All analyses were conducted at default settings.

Phage Silvanus has a siphophage morphology (Fig. 1). The 45,678-bp genome has a coding density of 97.4% and a G+C content of 58.4%. No tRNA genes were identified, and 26 out of 68 total genes were assigned putative functions, including a complete lysis cassette with genes encoding an endolysin of the glycosyl hydrolase class, a holin with three transmembrane domains and N-out, C-in topology (class I), and two-component spanins. Silvanus is predicted to use *cos*-type packaging because it encodes a

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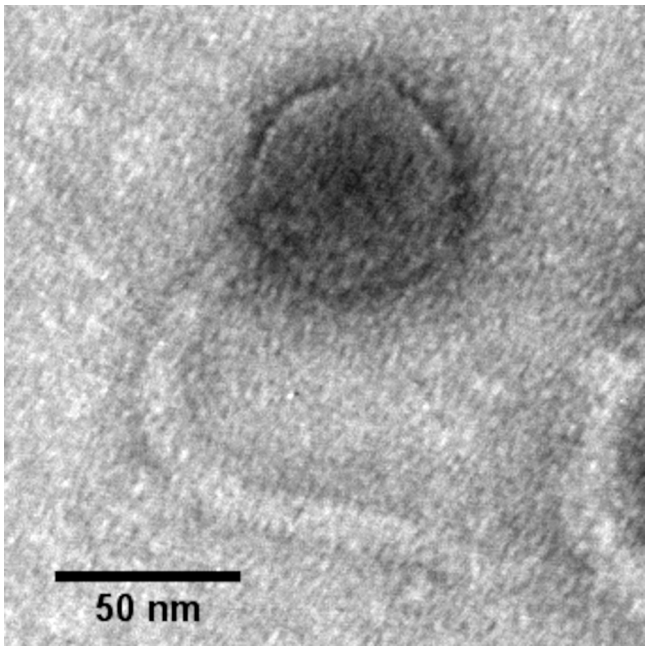

FIG 1 Transmission electron micrograph (TEM) of phage Silvanus. Phage particles were diluted with TEM buffer (20 mM NaCl, 10 mM Tris-HCl, pH 7.5, and 2 mM MgSO4) and captured on a freshly glow-discharged, Formvar carbon-coated grid. The grids were stained with 2% (wt/vol) uranyl acetate and observed on a JEOL 1200 EX TEM at 100 kV accelerating voltage at the Microscopy and Imaging Center at Texas A&M University.

large terminase subunit similar to that of the well-characterized *cos* phage HK97 (21% protein identity; E value, 10^{-8}; 100% HHpred probability) and also encodes an HNH endonuclease similar to that of HK97 gp74 (40% protein identity; E value, 10^{-21}) at the opposite end of the genome, which is required for the 3′ *cos* cleavage (22). Moreover, according to HHPred, the predicted small terminase has a 99.5% probability match to the structure of the *Pseudomonas* phage PaP3 small terminase, which generates cohesive ends (23). The precise location of phage Silvanus *cos* sites, however, cannot be determined by PhageTerm analysis. Whole-genome comparative genomics analysis by progressiveMauve v2.4 (21) revealed that Silvanus has <7% overall nucleotide identity to known phages. Silvanus was found to carry a T1 p38-like tail tape measure protein.

Data availability. The Silvanus genome was deposited in GenBank with accession number MZ326867. The associated BioProject, SRA, and BioSample accession numbers are PRJNA222858, SRR14095258, and SAMN18509682, respectively.

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