Complete Genome Sequence of *Stenotrophomonas maltophilia* Phage Philippe

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ABSTRACT *Stenotrophomonas maltophilia* is emerging as an opportunistic multidrug-resistant pathogen. *S. maltophilia* podophage Philippe has a 74,717-bp genome which is related broadly to the N4-like phage group, including *Stenotrophomonas* phage Pokken. The low sequence identity to other described phages suggests that Philippe is an unclassified member of the N4-like subfamily *Rothmandenesvirinae*.

The ubiquitous, Gram-negative bacterium *Stenotrophomonas maltophilia* plays an important role in beneficial plant interactions, functioning in both the sulfur and nitrogen cycles and pollutant degradation (1). The emergence of this species as an opportunistic, multidrug-resistant pathogen is a growing concern for immunocompromised patients, and phage therapy is being investigated to address this issue (2, 3). Here, the annotated genome sequence of *S. maltophilia* podophage Philippe is presented.

Phage Philippe was isolated in January 2019 from a soil sample in College Station, TX (GPS coordinates, 30.60322, −96.36004), using *S. maltophilia* (ATCC 17807) as the bacterial host. Soil (5 g) was mixed into 10 mL phosphate-buffered saline (PBS) buffer (pH 7.4), and the filtered supernatant (0.2-μm filter) was used for phage isolation. The host was cultured in tryptone nutrient broth (0.5% tryptone, 0.25% yeast extract, 0.1% glucose, 0.85% NaCl, wt/vol) at 30°C with aeration, and phage isolation and propagation were conducted via the soft agar overlay method (4, 5). Genomic DNA was purified from ~8 mL phage lysate using a modified Wizard DNA cleanup kit as previously described (6). DNA sequencing libraries were prepared as 300-bp inserts using a Swift 2S Turbo kit and sequenced on an Illumina MiSeq instrument with paired-end 150-bp reads using v2 300-cycle chemistry. The CPT Galaxy-Apollo phage annotation platform (https://cpt.tamu.edu/galaxy-pub) (7–9) was used for all subsequent analyses except HHpred (10). The 148,548 raw sequencing reads were quality controlled using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) and trimmed using the FASTX-Toolkit v0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/), prior to genome assembly using SPAdes v3.5.0 (11). A single contig was assembled with 94-fold coverage. The contig sequence was completed by PCR amplifying the end region using primers (5′-ATACCCGAGAACAGTGCAGC-3′ and 5′-CTATCTGGATCAGGCTGCCG-3′) and Sanger sequencing the resulting PCR product. Phage termini were predicted using PhageTerm (12). Gene predictions were made using Glimmer v3 (13) and MetaGeneAnnotator v1.0 (14). ARAGORN v2.36 (15) and tRNAscan-SE v2.0 (16) were used to detect tRNA genes. TransTermHP v2.09 was used for the identification of rho-independent termination sites (17). Gene functions were predicted using InterProScan v5.48 (18) and BLAST v2.9.0 (19) against the NCBI nonredundant and Swiss-Prot databases (20). Additional protein analysis was completed using TMHMM v2.0 (21), HHpred (10), LipoP v1.0 (22), and SignalP v5.0 (23). Genome-wide DNA sequence similarity was evaluated using ProgressiveMauve v2.4 (24). All analyses were conducted with default settings. The
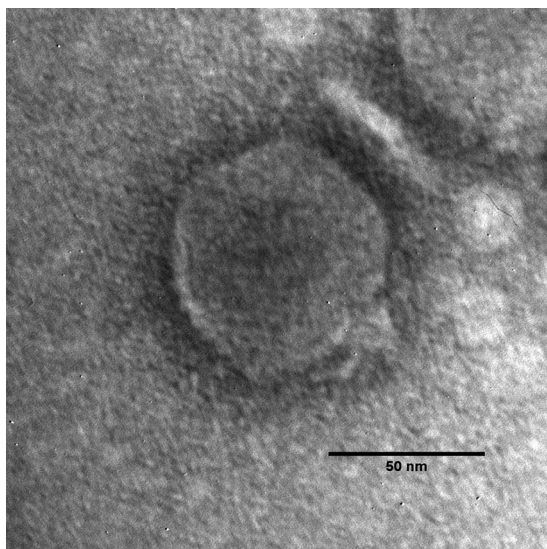
FIG 1 Transmission electron micrograph (TEM) of phage Philippe. Phage particles were diluted with TEM buffer (20 mM NaCl, 10 mM Tris-HCl [pH 7.5], 2 mM MgSO4) and captured on freshly glow-discharged, Formvar carbon-coated grids. The grids were stained with 2% (wt/vol) uranyl acetate and observed on a JEOL 1200 EX TEM instrument at 100 kV accelerating voltage at the Microscopy and Imaging Center at Texas A&M University.

phage morphology was determined by negatively staining the phage particles with 2% (wt/vol) uranyl acetate and observing them with transmission electron microscopy at the Texas A&M Microscopy and Imaging Center.

Phage Philippe has a podophage morphology (Fig. 1). It has a 74,717-bp genome with a GC content of 54.3%. A total of 94 protein-coding genes and 6 tRNAs were identified in the Philippe genome. Philippe is related broadly to the N4-like phage group, sharing the highest similarity to *Stenotrophomonas* phage Pokken (GenBank accession number NC_049463), with 26.4% nucleotide identity calculated using ProgressiveMauve and 50 similar proteins (BLASTp; E value, $<10^{-5}$). This low sequence identity to other described phages suggests that Philippe is an unclassified member of the N4-like subfamily *Rothmandenesvirinae*. All components of the lysis cassette in Philippe were identified, including the SAR endolysin *N*-acetylmuramidase, class II holin, and an o-spanin fully embedded within the i-spanin. As expected of an N4-like phage, three RNA polymerase (RNAP) genes were annotated, with the largest identified as the virion RNAP.

Data availability. The Philippe genome sequence was deposited in GenBank under accession number MZ326861. The associated BioProject, SRA, and BioSample accession numbers are PRJNA222858, SRR14095251, and SAMN18509471, respectively.

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