Complete Genome Sequence of Temperate *Stenotrophomonas maltophilia* Bacteriophage DLP5

Danielle L. Peters,[a] Jonathan J. Dennis[a]

[a]Department of Biological Sciences, University of Alberta, Edmonton, Canada

ABSTRACT *Stenotrophomonas maltophilia* bacteriophage DLP5 is a temperate phage with *Siphoviridae* family morphotype. DLP5 (vB_SmaS_DLP_5) is the first *S. maltophilia* phage shown to exist as a phagemid. The DLP5 genome is 96,542 bp, encoding 149 open reading frames (ORFs), including four tRNAs. Genomic characterization reveals moron genes potentially involved in host cell membrane modification.

*S*tenotrophomonas maltophilia* is an aerobic, opportunistic Gram-negative bacterium ubiquitous in aqueous environments, soils, plant rhizospheres, and hospital settings (1). *S. maltophilia* is capable of causing a variety of infections, and limited treatment options exist due to *S. maltophilia*'s exquisite innate multidrug resistance to a broad array of antibiotics (1–3). As an alternative to antibiotics, phages are being examined for treatment of *S. maltophilia* infections, with initial focus on phage isolation and characterization (4–16).

Phage DLP5 (vB_SmaS_DLP_5) was isolated from garden soil using *S. maltophilia* host strain D1614. Transmission electron micrographs identify DLP5 as a B1 morphotype *Siphoviridae* phage. DLP5 possesses relatively narrow tropism, infecting 5/27 clinical isolates tested. DLP5 forms clear plaques with defined boarders with an average size of 0.5 ± 0.2 mm, and one-step growth curves exhibit average burst sizes of 36. As a prophage, DLP5 replicates as a phagemid. Restriction fragment length polymorphism (RFLP) analysis suggests that the DNA is heavily modified; only 4/36 endonucleases tested could cut the DLP5 genome.

Genomic DNA was isolated from phage lysates using the Wizard DNA purification system and a modified protocol (17). A Nextera XT library was generated for paired-end sequencing on MiSeq (Illumina) platform using MiSeq v2 reagent kit and reads assembled using SPAdes 3.8.0 (18). The assembly was confirmed with PCR using 15 primer pairs randomly spaced throughout the genome with Sanger sequencing of PCR products. Open reading frames (ORFs) were identified using Glimmer (19) for Geneious (20) (Bacteria/Archaea setting) and Gene MarkS (21) for phage. The contig was annotated using BLASTP (22) and conserved domains were identified with CD-Search (23).

The phage DLP5 genome is 96,542 bp long (295-fold coverage), with GC content of 58.4%, and encoding 149 ORFs and 4 tRNAs (Sup-CTA, Glu-TTC, Gly-TCC, and Ser-GCT). Only 39 ORFs were classified with putative functions based on BLASTP analysis. DLP5 is predicted to encode two proteins involved in chromosome partitioning due to the presence of a ParBc superfamily domain; one ParBc protein also has a predicted SpoOJ superfamily domain. DNA replication, transcription, and repair proteins of interest include DNA polymerase I, DnaB, DnaG, superfamily II DNA/RNA helicase, DNA ligase, RecA, RuvC, RNase E, transcriptional regulator, transcriptional repressor, thymidylate synthase, phosphoglycerate kinase, UDP-glucose 4-epimerase, WcaG, tyrosine phosphatase, and pyruvate phosphate dikinase.

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Structure/packaging predicted proteins include portal protein, large terminase subunit, major capsid protein, three tail assembly proteins, a tail fiber protein, a tape measure protein, and lysozyme. DLP5 also encodes six moron genes, which are potential virulence factors, serine protease XkdF, SAM methyltransferase, rhomboid membrane protein, PIG-L family deacetylase, WecE, and an SPFH domain-containing protein. There are also three domain-of-unknown-function proteins encoded, DUF2500, DUF3310, and DUF1643. BLASTN analysis (22) shows DLP5 is relatively unrelated to other phages, exhibiting maximum similarity of 2% with *Xylella fastidiosa* phage Sano (24). DLP5 genome analysis provides insight into its characteristics and potential contributions to *S. maltophilia* hosts during lysogeny.

Accession number(s). The complete genomic sequence of *S. maltophilia* phage vB_SmaS_DLP_5 can be accessed in GenBank under the accession number MG189906.

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