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GENOME SEQUENCES

Genome Sequence of SN1, a Bacteriophage That Infects *Sphaerotilus natans* and *Pseudomonas aeruginosa*

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ABSTRACT Phage SN1 infects *Sphaerotilus natans* and *Pseudomonas aeruginosa* strains. Its genome consists of 61,858 bp (64.3% GC) and 89 genes, including 32 with predicted functions. SN1 genome is very similar to *Pseudomonas* phage M6, which contains hypermodified thymidines. Genome analyses revealed similar base-modifying genes as those found in M6.

Phage SN1 was isolated in 1979 from activated sludge samples obtained from a wastewater treatment plant (Lincoln, Nebraska, USA) using *S. natans* ATCC 13338 as the host (1, 2). An early study showed that the siphophage SN1 has unusual bases in its genome as confirmed by cellulose thin-layer chromatography (1). Its genomic DNA also showed resistance to type II restriction endonucleases (2). Host range studies indicate that phage SN1 can also infect *Pseudomonas aeruginosa* strains PAO33 and OT684 (2).

Here, phage SN1 was amplified with its host *S. natans* ATCC 13338 in nutrient broth (3 g/L beef extract, 5 g/L peptone) and agitated at 30°C (2). Cell debris were removed by filtration (0.45 μm) and filtrates were stored at 4°C until use. Phage SN1 also infected *P. aeruginosa* PAO1 (HER1153) in TSB/TSA medium at 30°C using both plaque assays and lysis of liquid cultures. Species identification of the above two host strains was confirmed by 16S sequencing.

Phage genomic DNA was purified from lysate (*S. natans* as host) using the phenol-chloroform extraction method (3). Library preparation for sequencing was carried out with Nextera XT DNA Sample Preparation kit (Illumina). A total of 186,025 paired-end reads (250 bp) were generated using the Illumina MiSeq Platform with Reagent kit v2. Read quality was evaluated with FastQC (4). Illumina adapters were removed and reads trimmed using Trimmomatic v0.39 (5). Trimmed reads assembly was performed by Spades assembler v3.13.0 (6). Two assemblies from independent lysates generated identical contigs of 61,858 nucleotides (218× and 170× coverage, respectively) with a GC content of 64.3%. Gene prediction and functional annotation were performed using RAST v2.0 (7) in combination with NCBI domain searches (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and blastp (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (8, 9) using NCBI non redundant and nucleotide databases. Comparisons with other phage genomes were carried out with NCBI blastn (8). Bioinformatic tools were run with default parameters.

Annotation of phage SN1 genome predicted 89 genes and 32 predicted functions, which included proteins involved in nucleotide synthesis modification, genome replication, structural proteins, and cell lysis. The top hits for similar genomes consisted of several *Pseudomonas* phages with 95 to 98% nucleotide identity (73 – 96% query cover). Interestingly, phage SN1 has 96.76% nucleotide identity (91% query cover) with *Pseudomonas* phage M6 genome, which contains hypermodified thymines (reviewed in reference [10]). Half of the thymine residues in the M6 genome contain moieties synthesized through postreplicative

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modifications of 5-hydroxymethyl uridine. In M6-like phages, including SN1, the thymidine modification pathway includes several genes located upstream of the DNA polymerase gene (10). This cassette consists of genes that code for pyrimidine hyroxymethylase (Locus tag SN1_071), Nmad5 (SN1_019), aGPT-Pplase1 (SN1_020), nucleotide kinase (SN1_021), rSAM (SN1_022), pyridoxal-5′-phosphate (PLP) dependent enzyme (SN1_023), and aGPT-Pplase2 (SN1_024). The hypermodified thymidines likely explain the resistance of the SN1 genome to certain type II endonucleases (2). Finally, we observed a gene that codes for a putative antirestriction protein (Locus tag SN1_075). These proteins typically mimic the DNA structure and block type I restriction enzymes (11–12).

Data Availability. Genome sequence is available under GenBank number ON165687. Raw sequence reads are available under SRA number SRR18758685. Phage SN1 is available at www.phage.ulaval.ca.

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