

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

---

Kenneth Nickerson Papers

Papers in the Biological Sciences

---

8-3-2022

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

K.M. Damitha Gunathilake

Denise M. Tremblay

Pier-Luc Plante

Ellen Jensen

Kenneth Nickerson

*See next page for additional authors*

Follow this and additional works at: <https://digitalcommons.unl.edu/bioscinickerson>



Part of the [Environmental Microbiology and Microbial Ecology Commons](#), [Other Life Sciences Commons](#), and the [Pathogenic Microbiology Commons](#)

---

This Article is brought to you for free and open access by the Papers in the Biological Sciences at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Kenneth Nickerson Papers by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

---

**Authors**

K.M. Damitha Gunathilake, Denise M. Tremblay, Pier-Luc Plante, Ellen Jensen, Kenneth Nickerson, and Sylvain Moineau



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

K. M. Damitha Gunathilake,<sup>a,b</sup> Denise M. Tremblay,<sup>b,d</sup> Pier-Luc Plante,<sup>c</sup> Ellen C. Jensen,<sup>e</sup> Kenneth W. Nickerson,<sup>e</sup>  Sylvain Moineau<sup>a,b,d</sup>

<sup>a</sup>Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval, Québec City, Québec, Canada

<sup>b</sup>Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Québec City, Québec, Canada

<sup>c</sup>Institute of nutrition and functional foods, Université Laval, Québec City, Québec, Canada

<sup>d</sup>Félix d'Hérelle Reference Center for Bacterial Viruses, Université Laval, Québec City, Québec, Canada

<sup>e</sup>School of Biological Sciences, University of Nebraska, Lincoln, NE, USA

**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

Published 3 August 2022

**Editor** Kenneth M. Stedman, Portland State University

**Copyright** © 2022 Gunathilake et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Sylvain Moineau, [Sylvain.Moineau@bcm.ulaval.ca](mailto:Sylvain.Moineau@bcm.ulaval.ca).

The authors declare no conflict of interest.

**Received** 11 May 2022

**Accepted** 12 July 2022

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 hydroxymethylase (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](#).

## ACKNOWLEDGMENTS

We thank Amanda Toperoff and Michi Waygood for editorial assistance.

This work was supported by the Human Frontier Science Program (grant RGP0024).

S.M. holds the Tier 1 Canada Research Chair in Bacteriophages.

## REFERENCES

1. Winston V, Thompson TL. 1979. Isolation and characterization of a bacteriophage specific for *Sphaerotilus natans* which contains an unusual base in its deoxyribonucleic acid. *Appl Environ Microbiol* 37:1025–1030. <https://doi.org/10.1128/aem.37.5.1025-1030.1979>.
2. Jensen EC, Schrader HS, Rieland B, Thompson TL, Lee KW, Nickerson KW, Kokjohn TA. 1998. Prevalence of broad-host-range lytic bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*. *Appl Environ Microbiol* 64:575–580. <https://doi.org/10.1128/AEM.64.2.575-580.1998>.
3. Moineau S, Pandian S, Klaenhammer TR. 1994. Evolution of a lytic bacteriophage via DNA acquisition from the *Lactococcus lactis* chromosome. *Appl Environ Microbiol* 60:1832–1841. <https://doi.org/10.1128/aem.60.6.1832-1841.1994>.
4. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
5. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
6. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
7. Aziz RK, Bartels D, Best AA, et al. 2008. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
8. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL. 2008. NCBI BLAST: a better web interface. *Nucleic Acids Res* 36:W5–W9. <https://doi.org/10.1093/nar/gkn201>.
9. Pruitt KD, Tatusova T, Maglott DR. 2005. NCBI Reference Sequence (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res* 33:D501–D504. <https://doi.org/10.1093/nar/gki025>.
10. Lee Y-J, Dai N, Müller SI, Guan C, Parker MJ, Fraser ME, Walsh SE, Sridar J, Mulholland A, Nayak K, Sun Z, Lin Y-C, Comb DG, Marks K, Gonzalez R, Dowling DP, Bandarian V, Saleh L, Corrêa IR, Weigle PR. 2021. Pathways of thymidine hypermodification. *Nucleic Acids Res* 50:3001–3017. <https://doi.org/10.1093/nar/gkab781>.
11. Bandyopadhyay PK, Studier FW, Hamilton DL, Yuan R. 1985. Inhibition of the type I restriction-modification enzymes EcoB and EcoK by the gene 0.3 protein of bacteriophage T7. *J Mol Biol* 182:567–578. [https://doi.org/10.1016/0022-2836\(85\)90242-6](https://doi.org/10.1016/0022-2836(85)90242-6).
12. Tock MR, Dryden DTF. 2005. The biology of restriction and anti-restriction. *Curr Opin Microbiol* 8:466–472. <https://doi.org/10.1016/j.mib.2005.06.003>.