Complete Genome Sequences of Genamy16 and NovaSharks, Two *Gordonia rubripertincta* Bacteriophages Isolated from Soil in Southeastern Florida

Julie Torruellas Garcia,a Sarah Ballarin,a Neel Balusa,b Melissa Bell,b Samia Caballero,a Joshua Chan,c Maria Farez,a Ashley Guillen-Tapia,a Kristin Parent,d Nashrah Pierre-Louis,a Victoria Polishuk,a Bhavya Soni,a Sundharraman Subramanian,d Katie Crumpa

aDepartment of Biological Sciences, Nova Southeastern University, Fort Lauderdale, Florida, USA
bDepartment of Psychology and Neuroscience, Nova Southeastern University, Fort Lauderdale, Florida, USA
cH. Wayne Huizenga College of Business and Entrepreneurship, Nova Southeastern University, Fort Lauderdale, Florida, USA
dDepartment of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan, USA

**ABSTRACT** We report on two actinobacteriophages, Genamy16 and NovaSharks, that were isolated from soil in Florida using *Gordonia rubripertincta* NRRL B-16540. The genomes of both phages are ~65,000 bp, with similar GC contents, and, based on gene content similarity to phages in the Actinobacteriophage Database, were assigned to phage cluster DV.

Bacteriophages (phages) have been used as a tool in many sectors to prevent bacterial growth. In the food industry, phages are applied to food as nonchemical means to prevent food spoilage by bacteria (1). With the rise of antimicrobial resistance, phage therapy is a promising alternative to conventional antibiotics (2). Additionally, phage treatment is being explored as a method for bioremediation of oil spills and wastewater treatment (3). Here, we report the discovery of two new phages, Genamy16 and NovaSharks, that infect *Gordonia rubripertincta*, a Gram-positive soil bacterium that can break down hydrocarbons (4).

Both phages were isolated using standard methods (5). Soil samples that had been collected at Nova Southeastern University (Davie, FL, USA) (Table 1) were washed in peptone-yeast-calcium (PYCa) medium, and the wash was then filtered (0.22-μm pore size). A fraction of the filtrate was plated in top agar with *Gordonia rubripertincta* NRRL B-16540 and incubated at 30°C, yielding phage Genamy16. The remaining filtrate was inoculated with *Gordonia rubripertincta* and incubated with shaking at 30°C for 5 days before being filtered and plated in top agar with *Gordonia rubripertincta*, yielding phage NovaSharks. Both bacteriophages were purified via three rounds of plating and exhibiting small, clear plaques (approximately 1.0 to 1.5 mm in diameter) after incubation at 30°C for 72 to 96 h. Negative-staining transmission electron microscopy demonstrated a *Siphoviridae* morphotype for both phages (Fig. 1A and B). The tail length and capsid diameter for each phage are shown in Table 1.

Genomic DNA was isolated using the Wizard DNA Clean-Up System (Promega). The Pittsburgh Bacteriophage Institute prepared sequencing libraries with the NEBNext Ultra II Library Kit (New England Biolabs). The libraries were run on an Illumina MiSeq instrument (v3 reagents). For each phage, the number of reads, read length, and coverage are shown in Table 1. Reads were assembled using Newbler v2.9 and quality checked for coverage and genome termini using Consed v29.0 as described previously (6, 7). Table 1 details the genomic characteristics for both phages. Genamy16 and NovaSharks were assigned to phage cluster DV based on content similarity of at least 35% to phages within the Actinobacteriophage Database (8, 9).

Both genome sequences were autoannotated using DNA Master v5.23.6 (10) embedded...
# TABLE 1

Sample isolation location, phage size, and genomic characteristics for Genamy16 and NovaSharks

| Phage name | Soil collection site coordinates | Capsid diam (nm) | Tail length (nm) | Sequencing read type | No. of reads | Avg sequencing coverage (×) | Cluster | Genome size (bp) | Genome ends | GC content (%) | No. of genes |
|------------|----------------------------------|------------------|------------------|----------------------|--------------|---------------------------|---------|-----------------|-------------|----------------|--------------|
| Genamy16   | 26.081101N, 80.243822W           | 63.8 ± 0.92 (n = 5) | 355.7 ± 12.3 (n = 5) | 150-base single end | 580,092      | 1,257                     | DV      | 65,574          | Circularly permuted | 57.6          | 97           |
| NovaSharks | 26.076111N, 80.243306W           | 65.2 ± 1.44 (n = 5) | 363.9 ± 5.39 (n = 5) | 150-base single end | 464,243      | 1,017                     | DV      | 65,274          | Circularly permuted | 57.7          | 96           |
with GeneMark v2.5 (11) and Glimmer v3.02 (12) and then were manually refined using Starterator v474 (8). Gene function was assessed with HHpred (13, 14), NCBI BLASTp (15), and SOSUI (16). tRNA genes were assessed with tRNAscan-SE v2.0 (17) and ARAGORN v1.2.38 (18). All software was used with default parameters. No tRNAs were identified in either phage.

The numbers of predicted genes for both phages are detailed in Table 1. All genes are transcribed rightward. The two phages exhibit similar gene organization, with the left arm of the genome containing structure and assembly genes such as the portal protein, minor and major capsid proteins, head-to-tail stopper, tail terminator, major tail protein, and tape measure protein, followed by several minor tail proteins. The right arm of the genome encodes proteins for DNA metabolism functions, including DNA helicase, RecA-like DNA recombinase, oxidoreductase, DnaE-like polymerase III, MazG-like protein, thymidylate synthase, and resolvase.

**Data availability.** The sequence of Genamy16 is available in GenBank with accession no. ON755185 and Sequence Read Archive (SRA) accession no. SRX14443507. The sequence of NovaSharks is available in GenBank with accession no. ON755187 and SRA accession no. SRX14483224.

**ACKNOWLEDGMENTS**

This study was supported by the Department of Biological Sciences, Halmos College of Arts and Sciences at Nova Southeastern University, the Howard Hughes Medical Institute Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program, and the University of Pittsburgh. S.S. was supported by National Science Foundation Career Award 1750125.

We thank Viknesh Sivanathan, senior program lead at the Howard Hughes Medical Institute, for his assistance in extracting the phage DNA. We also thank the Michigan State University Research Technology Support Facility (RTSF) Cryo-EM Core Facility for the use of the Talos Arctica system.

**REFERENCES**

1. Polaska M, Sokolowska B. 2019. Bacteriophages—a new hope or a huge problem in the food industry. AIMS Microbiol 5:324–346. [https://doi.org/10.3934/microbiol.2019.4.324](https://doi.org/10.3934/microbiol.2019.4.324).

2. Hatfull GF, Dedrick RM, Schooley RT. 2022. Phage therapy for antibiotic-resistant bacterial infections. Annu Rev Med 73:197–211. [https://doi.org/10.1146/annurev-med-080219-122208](https://doi.org/10.1146/annurev-med-080219-122208).
3. Cristobal-Cueto P, Garcia-Quintanilla A, Esteban J, Garcia-Quintanilla M. 2021. Phages in food industry biocontrol and bioremediation. Antibiotics (Basel) 10:786. https://doi.org/10.3390/antibiotics10070786.

4. Sowani H, Kulkarni M, Zinjarde S. 2018. An insight into the ecology, diversity and adaptations of Gordonia species. Crit Rev Microbiol 44:393–413. https://doi.org/10.1080/1040841X.2017.1418286.

5. Poxleitner M, Pope W, Jacobs-Sera D, Sivanathan V, Hatfull G. 2018. Phage discovery guide. Howard Hughes Medical Institute, Chevy Chase, MD. https://seaphagesphagediscoveryguide.helpdocsonline.com/home.

6. Gordon D, Green P. 2013. Consed: a graphical editor for next-generation sequencing. Bioinformatics 29:2936–2937. https://doi.org/10.1093/bioinformatics/btt515.

7. Russell DA. 2018. Sequencing, assembling, and finishing complete bacteriophage genomes. Methods Mol Biol 1681:109–125. https://doi.org/10.1007/978-1-4939-7343-9_9.

8. Russell DA, Hatfull GF. 2017. PhagesDB: the Actinobacteriophage Database. Bioinformatics 33:784–786. https://doi.org/10.1093/bioinformatics/btw711.

9. Pope WH, Mavrich TN, Garlена RA, Guerrero-Bustamante CA, Jacobs-Sera D, Montgomery MT, Russell DA, Warner MH, Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES), Hatfull GF. 2017. Bacteriophages of Gordonia spp. display a spectrum of diversity and genetic relationships. mBio 8:e01069-17. https://doi.org/10.1128/mBio.01069-17.

10. Pope WH, Jacobs-Sera D. 2018. Annotation of bacteriophage genome sequences using DNA Master: an overview. Methods Mol Biol 1681:217–229. https://doi.org/10.1007/978-1-4939-7343-9_16.

11. Besemer J, Borodovsky M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Res 33:W451–W454. https://doi.org/10.1093/nar/gki487.

12. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679. https://doi.org/10.1093/bioinformatics/btm009.

13. Gabler F, Nam SZ, Till S, Mirdita M, Steinegger M, Söding J, Lupas AN, Alva V. 2020. Protein sequence analysis using the MPI Bioinformatics Toolkit. Curr Protoc Bioinformatics 72:e108. https://doi.org/10.1002/cpbi.108.

14. Zimmermann L, Stephens A, Nam SZ, Rau D, Kübler J, Lozajic M, Gabler F, Söding J, Lupas AN, Alva V. 2018. A completely reimplemented MPI Bioinformatics Toolkit with a new HHpred server at its core. J Mol Biol 430: 2237–2243. https://doi.org/10.1016/j.jmb.2017.12.007.

15. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

16. Hirokawa T, Boon-Chieng S, Mitaku S. 1998. SOSUI: classification and secondary structure prediction system for membrane proteins. Bioinformatics 14:378–379. https://doi.org/10.1093/bioinformatics/14.4.378.

17. Lowe TM, Chan PP. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44:W54–W57. https://doi.org/10.1093/nar/gkw413.

18. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16. https://doi.org/10.1093/nar/gkh152.