| Bacteriophages | Announcement

Complete genome sequences of seven Microbacterium foliorum phages Albedo, Kenzers, Swervy, Cranjis, JaimeB, Fullmetal, and Stormbreaker

Keyshla Valentin Caban,1 Elizabeth Kalesnik,1 Kaitlyn A. Green,1 Christopher J. Negro,1 Ulises Nunez Rodriguez,1 Milan C. Peele,1 Casandra T. Nguyen,1 Sydney Cahill,1 Keara Dougherty,1 Melissa Logue,1 Star Hargraves,1 Hannah Radziak,1 Luke Willette,1 Esther Ogunyinka,1 Davia C. Campbell,1 Oluwatomiwa Adebamiro,1 Cecelia Schmeltzer,1 Jonathan Onimus,1 Hannah A. Asaka,1 Wilfred Bangura,1 Christina M. Shimp,1 Ameera Alade,1 Daekwon M. Sequira,1 Tommy Jimenez,1 Neumann University Phage Discovery Group, Sarah J. Swerdlow,2 Melinda K. Harrison,3 Patricia C. Fallest-Strobl,1 Matthew D. Mastropaolo1

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT Seven bacteriophages were isolated from soil in Pennsylvania and Wisconsin using the host Microbacterium foliorum. These bacteriophages range in the number of predicted genes encoded, from 25 to 91, and are distributed across actinobac teriophage clusters EB, EC, EE, and EK.

KEYWORDS bacteriophages, genomics, cluster, DNA sequencing

Bacteriophages are incredibly abundant and genetically diverse. To expand our knowledge of bacteriophage evolution and diversity, we report here the characteris tics of seven bacteriophages newly isolated using Microbacterium foliorum NRRL B-24224 (1, 2).

All seven bacteriophages were isolated from soil in Pennsylvania and Wisconsin using standard methods as previously described (Table 1) (3, 4). These soil samples were incubated in peptone-yeast extract-calcium (PYCa) liquid medium for 2 hours at 30°C with shaking to suspend phage particles. The suspension was then filtered through a 0.22-µm filter. The filtrate was either directly plated in PYCa soft agar containing M. foliorum or “enriched” by inoculation with M. foliorum and incubation at 30°C for 2–3

days before being filtered and plated (Table 1), yielding phages Albedo, Kenzers, Swervy, Cranjis, JaimeB, Fullmetal, and Stormbreaker. All phages were purified through three rounds of plating. All plates were incubated at 30°C for 24–48 hours.

The Wizard DNA Cleanup Kit (Promega) was used to extract genomic DNA from phage lysates, as previously described (4). Some lysates were concentrated using ZnCl2 precipitation prior to genomic DNA extraction (6). The genomic DNA libraries were prepared using a NEBNext Ultra II FS Kit (New England BioLabs) followed by sequencing using Illumina MiSeq (v3 reagents), yielding at least 40,000 150-base single-end reads (Table 1). Raw reads were assembled and then checked for completeness using Newbler v2.9 (7) and Consed v29 (8), respectively (9). Sequencing results and genome characteris tics of each bacteriophage are listed in Table 1.

The genomes were autoannotated using DNA Master v5.23.6 (http://coba mide2.bio.pitt.edu), Glimmer v3.02b (10), GeneMark v4.28 (11) and were refined using PECAAN v20221109 (https://pecaan.kbrinsgd.org/index.html), Starterator v462 (https:// github.com/SEA-PHAGES/starterator), and Phamerator v539 (12). Transmembrane helices were predicted using SOSUI v1.11 (13), TOPCONS v2.0 (14), TMHMM v2.0 (15), and DeepTMHMM v1.0.24 (16). tRNAs were predicted using ARAGORN v1.2.41 (17) and tRNAscanSE v2.0 (18). Putative functions for other predicted genes were made using

Editor Simon Roux, DOE Joint Genome Institute,
USA

Address correspondence to Matthew D. Mastropaolo, mastropm@neumann.edu.

The authors declare no conflict of interest.

Received 19 December 2023

Accepted 16 February 2024

Published 7 March 2024

10.1128/mra.01251-23 1

April 2024 Volume 13 Issue 4

TABLE 1 Bacteriophage, plaque morphology, and genomic characteristics

Phage name	Soil sample collection site	Isolation method	Plaque morphology	Plaque Sizea (mm)	Approx. shotgun coverage (fold)	No. of 150-bp single-end reads	Genome length (bp)	Genome end characteristic	G + C content (%)	No. of ORFsb	No. of tRNAs	Clusterc
Albedo	Hudson, WI, 44.984533 N, 92.7545 W	Enriched	Clear with halo	0.1–1	83	862,002	41,813	3' single-stranded overhang				

5'-TCTCCCGGCA-3'

66.6 71 1 (Gln) EB

Kenzers Greenville, PA, 41.4124 N,

80.3813 W

Enriched Clear 1 3,293 960,944 41,261 3' single-stranded overhang

5'-TCTCCCGGCA-3'

66.8 70 1 (Gln) EB

Swervy Aston, PA, 39.8657 N, 75.4279 W Direct Turbid 0.5–1 325 93,814 41,510 3' single-stranded overhang

5'-TCTCCCGGCA-3'

66.7 71 1 (Asn) EB

Cranjis Upper Chichester, PA, 39.856232 N,

75.443149 W

Direct Turbid 3.5–4 99 40,843 53,222 Circularly permutated 68.9 91 0 EC

JaimeB Aston, PA,

39.875331 N, 75.440021 W

Direct Clear 3–4 14,635 1.8 million 17,445 3' single-stranded overhang

5'-CCCGCCCCA-3'

68.7 25 0 EE

Stormbreaker Aston, PA, 39.5215 N, 75.260806 W Direct Clear 1 1,073 74,440 54,050 Circularly permutated 60 54 0 EKe

Fullmetal Aston, PA, 39.876667 N, 75.441667 W Direct Clear 1–1.5 197 410,171 54,438 Circularly permutated 59.8 55 0 EKf

aPlaque size is based on the measurements of three plaques.

bORFs, open reading frames.

cClusters were identified using sequence similarities to other Microbacterium phage (5).

dIndicates a clear middle of the plaque with a diffuse or cloudy edge.

eSubcluster EK2.

fSubcluster EK1.

g"ND” indicates that the TEM was not performed.

hIsolation methods are described in the Phage Discovery Guide (3, 4).

April 2024 Volume 13 Issue 4 10.1128/mra.01251-23 2

Announcement

Microbiology Resource Announcements

HHPRED v3.2 (against the PDB_mmCIF70, NCBI_Conserved_Domains, Pfam-A, and UniProt-SwissProt databases) (19) and BlastP v2.10.0 (against the PhagesDB and NCBI nonredundant databases) (20). All annotations were performed with default parameters.

Phages were assigned to clusters based on gene content similarity (GCS) of at least 35% to sequenced genomes in the Acinobacteriophage database (https://phagesdb.org/) using the GCS tool at phagesDB (5, 21). All seven phages reported here are consistent with features previously described for their respective clusters; the EB cluster phages, Albedo, Kenzers, and Swervy encode for <3 tRNAs; the EC cluster phage Cranjis has all its genes transcribed rightward; the EE cluster phage JaimeB shares all 25 predicted genes including a capsid maturation and protease fusion protein with the other EE cluster members; the EK cluster phages Stormbreaker and Fullmetal have the f

irst ~30 predicted genes transcribed leftward and all the remaining genes transcribed rightward, and they also encode for the largest actinobacteriophage gene product, over 4,400 amino acids (1, 22).

ACKNOWLEDGMENTS

We thank Dan Russell for sequencing and assembly of the bacteriophages in this study. We also thank Steven Cresawn, Debbie Jacobs-Sera, Graham Hatfull, Viknesh Sivanathan, and other members of the SEA-PHAGES and Howard Hughes Medical Institute for their expertise and support of our research.

AUTHOR AFFILIATIONS

1

Sciences, Neumann University, Aston, Pennsylvania, USA

2Biological Sciences, University of Pittsburgh Greensburg, Greensburg, Pennsylvania, USA

3

Sciences, Cabrini University, Radnor, Pennsylvania, USA

AUTHOR ORCIDs

Melinda K. Harrison

http://orcid.org/0000-0003-2047-7087

Matthew D. Mastropaolo

AUTHOR CONTRIBUTIONS

http://orcid.org/0000-0002-8148-4742

Keyshla Valentin Caban, Investigation, Validation | Elizabeth Kalesnik, Investigation, Validation | Kaitlyn A. Green, Investigation, Validation | Christopher J. Negro, Investiga tion, Validation | Ulises Nunez Rodriguez, Investigation, Validation | Milan C. Peele, Investigation, Validation | Casandra T. Nguyen, Investigation, Validation | Sydney Cahill, Investigation, Validation | Keara Dougherty, Investigation, Validation | Melissa Logue, Investigation, Validation | Star Hargraves, Investigation, Validation | Hannah Radziak, Investigation, Validation | Luke Willette, Investigation, Validation | Esther Ogunyinka, Investigation, Validation | Davia C. Campbell, Investigation, Validation | Oluwatomiwa Adebamiro, Investigation, Validation | Cecelia Schmeltzer, Investigation, Validation | Jonathan Onimus, Investigation, Validation | Hannah A. Asaka, Investigation, Validation | Wilfred Bangura, Investigation, Validation | Christina M. Shimp, Investigation, Validation | Ameera Alade, Investigation, Validation | Daekwon M. Sequira, Investigation, Validation | Tommy Jimenez, Investigation, Validation | Sarah J. Swerdlow, Validation, Writing – review and editing | Melinda K. Harrison, Data curation, Investigation, Validation, Writing – review and editing | Patricia C. Fallest-Strobl, Data curation, Investigation, Validation | Matthew D. Mastropaolo, Conceptualization, Data curation, Formal analysis, Investiga tion, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review and editing.

DATA AVAILABILITY

All genomes, Albedo, Kenzers, Swervy, Cranjis, JaimeB, Fullmetal, and Stormbreaker are available at GenBank with Accession No. OR475283, OP172875, MZ747513,

April 2024 Volume 13 Issue 4

10.1128/mra.01251-23 3

Microbiology Resource Announcements

Announcement

OP297543, OR195050, OP297538, MT657334 and the Sequence Read Archive (SRA) No. SRX22868877, SRX14483228, SRX14485092, SRX22853654, SRX22853656, SRX22853655, SRX22853658, respectively.

REFERENCES

1.

2.

3.

4.

5.

6.

7.

8.

9.

Mastropaolo MD, Fallest-Strobl PC, Sequira DM, Campbell DD, Negro CJ, Tuang KS, Sigmund-Hamre IM, Womack CL, Mansbridge T, Metzler AL, Sasher EG, Collins C, Crowley NK, Dower VR, Bates M, Bjorkelo C, Johnson H, Salvitti LR, Neumann University Phage Discovery Group. 2023. Genome sequences of five Microbacterium foliorum phages, GaeCeo, NeumannU, Eightball, Chivey, and Hiddenleaf. Microbiol Resour Announc 12:e0110622. https://doi.org/10.1128/mra.01106-22

Hatfull GF. 2020. Actinobacteriophages: genomics, dynamics, and

applications. Annu Rev Virol 7:37–61. https://doi.org/10.1146/annurev
virology-122019-070009
Jordan TC, Burnett SH, Carson S, Caruso SM, Clase K, DeJong RJ,
Dennehy JJ, Denver DR, Dunbar D, Elgin SCR, et al. 2014. A broadly
implementable research course in phage discovery and genomics for
f
irst-year undergraduate students. mBio 5:e01051-13. https://doi.org/10.
1128/mBio.01051-13
Poxleitner M, Pope W, Jacobs-Sera D, Sivanathan V, Hatfull G. 2018.
Phage discovery guide. Howard Hughes Medical Institute, Chevy Chase,
MD. Available from:
helpdocsonline.com/home
Russell DA, Hatfull GF. 2017. PhagesDB: the actinobacteriophage
database.
Bioinformatics
bioinformatics/btw711
Santos MA. 1991. An improved method for the small scale preparation of
bacteriophage DNA based on phage precipitation by zinc chloride.
Nucleic Acids Res 19:5442. https://doi.org/10.1093/nar/19.19.5442
Miller JR, Koren S, Sutton G. 2010. Assembly algorithms for next
generation sequencing data. Genomics 95:315–327. https://doi.org/10.
1016/j.ygeno.2010.03.001
Gordon D, Green P. 2013. Consed: a graphical editor for next-generation
sequencing. Bioinformatics 29:2936–2937. https://doi.org/10.1093/
bioinformatics/btt515
Russell DA. 2018. Sequencing, assembling, and finishing complete
bacteriophage genomes. Methods Mol Bio 1681:109–125. https://doi.
org/10.1007/978-1-4939-7343-9
10. 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
11. Besemer J. Borodovsky M. 2005. Genemark: web software for gene
f
inding in prokaryotes, eukaryotes and viruses. Nucleic Acids Res
33:W451–W454. https://doi.org/10.1093/nar/gki487.13
12. Cresawn SG, Bogel M, Day N, Jacobs-Sera D, Hendrix RW, Hatfull GF.
2011. Phamerator: a bioinformatic tool for comparative bacteriophage
genomics. BMC Bioinformatics 12:395. https://doi.org/10.1186/1471
2105-12-395
13. 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
14. Tsirigos KD, Peters C, Shu N, Käll L, Elofsson A. 2015. The TOPCONS web
server for consensus prediction of membrane protein topology and
signal peptides. Nucleic Acids Res 43:W401–W407. https://doi.org/10.
1093/nar/gkv485
15. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. 2001. Predicting
transmembrane protein topology with a hidden markov model:
application to complete genomes. J Mol Biol 305:567–580. https://doi.
org/10.1006/jmbi.2000.4315
https://seaphagesphagediscoveryguide.
33:784–786.
https://doi.org/10.1093/
16. Hallgren J, Tsirigos KD, Pedersen MD, Almagro Armenteros JJ, Marcatili P,
Nielsen H, Krogh A, Winther O. 2022. Deeptmhmm predicts alpha and
beta transmembrane proteins using deep neural networks. bioRxiv.
https://doi.org/10.1101/2022.04.08.487609

17. 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

18. 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

19. 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

20. Söding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Res 33:W244–W248. https://doi.org/10.1093/nar/gki408

21. Pope WH, Mavrich TN, Garlena RA, Guerrero-Bustamante CA, Jacobs Sera D, Montgomery MT, Russell DA, Warner MH, Hatfull GF, Science Education Alliance-Phage Hunters Advancing Genomics and Evolution ary Science (SEA-PHAGES). 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

22. Jacobs-Sera D, Abad LA, Alvey RM, Anders KR, Aull HG, Bhalla SS, Blumer LS, Bollivar DW, Bonilla JA, Butela KA, et al. 2020. Genomic diversity of bacteriophages infecting Microbacterium spp. PLoS One 15:e0234636. https://doi.org/10.1371/journal.pone.023463