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Genomic sequences of *Mycobacterium smegmatis* A cluster phages LBerry, Pembroke, and Zolita

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ABSTRACT LBerry, Pembroke, and Zolita are newly isolated bacteriophages that infect *Mycobacterium smegmatis* mc^{2}155. Based on gene content similarity, LBerry and Pembroke are assigned to cluster A3, and Zolita is assigned to cluster A5. LBerry and Pembroke are 99% identical to Anaysia and Caviar, and Zolita is 99% identical to SydNat.

KEYWORDS bacteriophage, mycobacteria, genome analysis

The Mycobacterium genus of bacteria includes increasingly antibiotic-resistant human pathogens, such as *Mycobacterium tuberculosis* and *Mycobacterium abscessus* (1). With the increasing occurrence of antibiotic-resistant pathogens constituting a global threat to public health, phage therapy has recently been employed as an alternative treatment strategy. Some phages isolated using the nonpathogenic bacterial host *M. smegmatis* also infect pathogenic *Mycobacteria* and these phages can potentially be used in phage therapy (2, 3).

LBerry, Pembroke, and Zolita were isolated, purified, and their genomes were annotated through our participation in the SEA PHAGES program (4). Plaque purification, amplification, and production of high-titer lysates were performed as described in the Phage Discovery Guide (5).

All three phages were isolated from damp grassy soil samples collected in the northeastern US; with LBerry isolated outside of a hotel, Pembroke from a former farm, and Zolita near a flower bed. Each sample was treated with 7H9 liquid medium, filtered (0.2 µm), and inoculated with *Mycobacterium smegmatis* mc^2 155. Samples were incubated at 37°C with shaking for 48 h, then plated on top agar with host bacteria to form plaques. Three rounds of purification were done for LBerry and Pembroke, and four rounds for Zolita. All three phages were determined to have siphovirus morphology via negative-strain transmission electron microscopy (Fig. 1). DNA from each phage was extracted from a high-titer lysate by phenol: chloroform: isoamyl: alcohol extraction (6) and sequenced by the Pittsburgh Bacteriophage Institute (Table 1). Raw reads were verified for accuracy using Consed v29.0 (7) and assembled using Newbler v2.9 (8). All phage genomes have a 3′ single-stranded overhang; the sequences are reported in Table 1 along with genome sizes and GC content for each phage. Based on gene sequence similarities, LBerry and Pembroke were assigned to the A3 cluster while Zolita was assigned to the A5 cluster (9, 10). LBerry and Pembroke are 99% identical to A3 cluster phages Anaysia OP021679 and Caviar ON970623 (11), and Zolita is 99% identical to A5 phage SydNat ON970625.

DNA Master v5.23.6 was used to perform the genome annotations (12). GeneMark v2.5 (13), Glimmer v3.02 (14), and Starterator v.546 (15) were used to determine gene starts. Protein functions were determined using HHpred (PDB, UniProt, Pfam-A v.36, and NCBI v.3.19 databases) (16, 17), BLASTp v.2.14.1 (18), and Phamerator (19). ARAGORN v.1.2.38 (20) and tRNAscan-SE v.2.0 (21) were used to identify tRNAs. Membrane proteins

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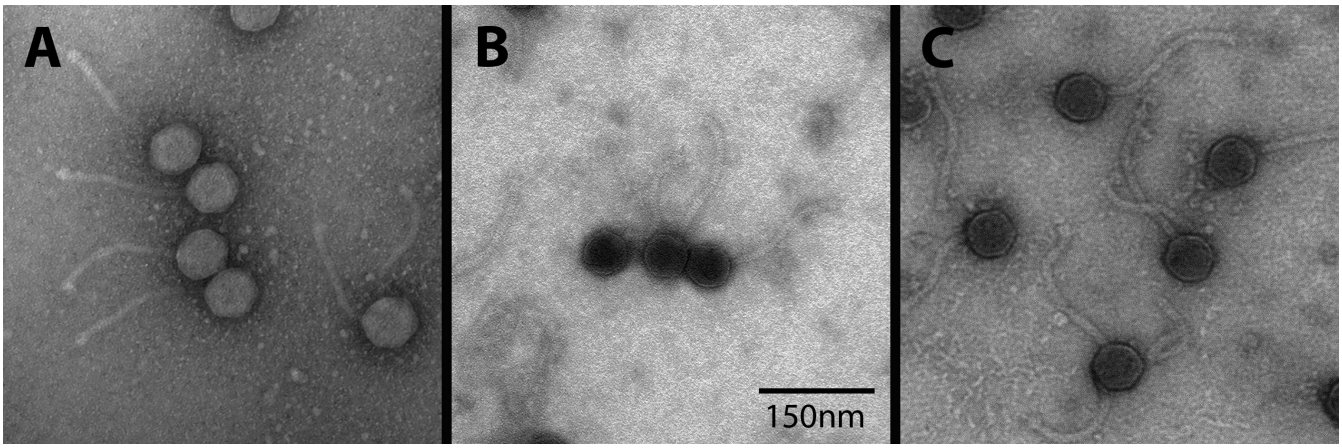

FIG 1 Images of (A) LBerry, (B) Pembroke, and (C) Zolita in negative-stained (1% uranyl acetate) taken by a JEOL 200 CX transmission electron microscope. All three phages have siphovirus morphology.

were predicted using TMHMM v.1.0.24 (22) and TOPCONS v.2.0 (23). Unless otherwise stated, default parameters were used for the programs listed.

Cluster A is the largest group of mycobacteriophages, with nearly 800 members. They are genetically diverse (24), and divided into 20 subclusters. LBerry, Pembroke, and Zolita follow the expected synteny of an A cluster phage beginning with a lysis cassette followed by structural proteins, integration proteins, replication/recombination proteins, an immunity repressor, and ending with a series of proteins of unknown function. The presence of immunity repressor and integrase genes in all three phages suggests that these phages could potentially adopt a temperate lifestyle (25). It has been determined that A3 cluster phages are able to infect *M. tuberculosis* H37Rv (26), indicating that LBerry and Pembroke could be further investigated for application in phage therapy.

TABLE 1 Sequencing, genome, and phage characteristics

Parameter	LBerry	Pembroke	Zolita
Soil sample characteristics			
Collection date	17 October 2022	10 October 2022	29 August 2018
Collection location coordinates	43.058056 N	42.076111 N	41.843056 N
	77.650556 W	70.833056 W	71.438611 W
Phage particle characteristics			
Capsid size (nm)	68–71 (n = 20)	59–63 (n = 20)	67–69 (n = 20)
Tail length (nm)	184–187 (n = 20)	189–192 (n = 20)	211–214 (n = 20)
Taxonomic identification			
Class	*Caudoviricetes*		
Genus	*Microwolfvirus*		*Benedictvirus*
Species	Unclassified		*Benedictvirus Zolita*
Sequencing			
Sequencing instrument	Illumina MiSeq v3 reagents		
Library prep kit	TruSeq DNA Nano Prep, S4 Flowcell, v1.5	NEB Ultra II Library Kit	
Number of reads	100,000	100,000	552,393
Length of reads (bp)	150-base single-end reads		
Shotgun coverage (×)	276	280	1,523
Phage genome characteristics			
Genome length (bp)	50,965	50,849	51,182
3′ single-stranded overhang sequence	CGGGTGGTAA	CGGGTGGTAA	CGGGAGGTAA
GC content (%)	64.0%	64.0%	60.9%

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DATA AVAILABILITY

The genome sequence accession number for LBerry is OR725491 and the SRA accession number is SRX23702564. The genome accession number for Pembroke is OR725495 and the SRA accession number is SRX23702567. The genome accession number for Zolita is MN096372 and the SRA accession number is SRX18224444.

REFERENCES

1. Hatfull GF, Hendrix RW. 2011. Bacteriophages and their genomes. Curr Opin Virol 1:298–303. https://doi.org/10.1016/j.coviro.2011.06.009
2. Nobrega FL, Costa AR, Kluskens LD, Azeredo J. 2015. Revisiting phage therapy: new applications for old resources. Trends Microbiol. 23:185–191. https://doi.org/10.1016/j.tim.2015.01.006
3. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, Russell DA, Ford K, Harris K, Gilmour KC, Soothill J, Jacobs-Sera D, Schooley RT, Hatfull GF, Spencer H. 2019. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. Nat Med 25:730–733. https://doi.org/10.1038/s41591-019-0437-z
4. 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 first-year undergraduate students. mBio 5:e01051-13. https://doi.org/10.1128/mBio.01051-13
5. Poxleitner: phage discovery guide. Google scholar. Available from: https://scholar.google.com/scholar_lookup?title=Phage+discovery+guide&publication_year=2018&;. Retrieved 6 Mar 2024.
6. Spada S. 2020. Chapter six - methods to purify DNA from extracellular vesicles: focus on exosomes, p 109–118. In Spada S, Galluzzi L (ed), Methods in enzymology. Academic Press.
7. Russell DA. 2018. Sequencing, assembling, and finishing complete bacteriophage genomes, p 109–125. In Clokie MRJ, Kropinski AM,

Lavigne R (ed), Bacteriophages: methods and protocols. Vol. 3. Springer, New York, NY.
8. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen Y-J, Chen Z, et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380. https://doi.org/10.1038/nature03959
9. Hatfull GF. 2014. Molecular genetics of mycobacteriophages. Microbiol Spectr 2. https://doi.org/10.1128/microbiolspec.MGM2-0032-2013
10. Russell DA, Hatfull GF. 2017. PhagesDB: the actinobacteriophage database. Bioinformatics 33:784–786. https://doi.org/10.1093/bioinformatics/btw711
11. Abidin ZU, Aucapina JE, Beauzil S, Berotte CM, Bonsu AO, Burgos GY, Chak STC, Collymore A, Daley ER, Defarias R, et al. 2022. Complete genome sequences of actinobacteriophages Anaysia and Caviar. Microbiol Resour Announc 11:e0094422. https://doi.org/10.1128/mra.00944-22
12. Pope WH, Jacobs-Sera D. 2018. Annotation of bacteriophage genome sequences using DNA master: an overview, p 217–229. In Clokie MRJ, Kropinski AM, Lavigne R (ed), Bacteriophages: methods and protocols. Vol. 3. Springer, New York, NY.
13. Lukashin AV, Borodovsky M. 1998. GeneMark.hmm: new solutions for gene finding. Nucleic Acids Res. 26:1107–1115. https://doi.org/10.1093/nar/26.4.1107
14. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res. 27:4636–4641. https://doi.org/10.1093/nar/27.23.4636
15. Pacey M. 2016. Starterator guide. University of Pittsburgh, Pittsburgh, PA.
16. Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Geer LY, Bryant SH. 2017. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. Nucleic Acids Res 45:D200–D203. https://doi.org/10.1093/nar/gkw1129
17. 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
18. 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
19. 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
20. 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
21. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25:955–964. https://doi.org/10.1093/nar/25.5.955
22. Möller S, Croning MDR, Apweiler R. 2001. Evaluation of methods for the prediction of membrane spanning regions. Bioinformatics 17:646–653. https://doi.org/10.1093/bioinformatics/17.7.646
23. 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
24. Mavrich TN, Hatfull GF. 2019. Evolution of superinfection immunity in cluster A mycobacteriophages. mBio 10:e00971-19. https://doi.org/10.1128/mBio.00971-19
25. Mavrich TN, Hatfull GF. 2017. Bacteriophage evolution differs by host, lifestyle and genome. Nat Microbiol 2:17112. https://doi.org/10.1038/nmicrobiol.2017.112
26. Guerrero-Bustamante CA, Dedrick RM, Garlena RA, Russell DA, Hatfull GF. 2021. Toward a phage cocktail for tuberculosis: susceptibility and tuberculocidal action of mycobacteriophages against diverse *Mycobacterium tuberculosis* strains. mBio 12:e00973-21. https://doi.org/10.1128/mBio.00973-21