

6-20-2019

Discovery and Characterization of Bacteriophage LuckyBarnes

Savannah L. Underwood
University of Southern Mississippi

Amanda Foto
University of Southern Mississippi

Amanda F. Ray
University of Southern Mississippi

Alexander E. Nelms
University of Southern Mississippi

Keyshawn L. Kennedy
University of Southern Mississippi

See next page for additional authors

Follow this and additional works at: https://aquila.usm.edu/fac_pubs

 Part of the [Biology Commons](#)

Recommended Citation

Underwood, S. L., Foto, A., Ray, A. F., Nelms, A. E., Kennedy, K. L., Hartley, S. G., Ryals, L. M., Gurung, C., D'Angelo, W. A., Pope, W. H., Mavrodi, D. V. (2019). Discovery and Characterization of Bacteriophage LuckyBarnes. *Microbiology Resource Announcements*(25), 1-2.

Available at: https://aquila.usm.edu/fac_pubs/16424

Authors

Savannah L. Underwood, Amanda Foto, Amanda F. Ray, Alexander E. Nelms, Keyshawn L. Kennedy, Shelby G. Hartley, Logan M. Ryals, Chandan Gurung, William A. D'Angelo, Welkin H. Pope, and Dmitri V. Mavrodi



Discovery and Characterization of Bacteriophage LuckyBarnes

Savannah L. Underwood,^a Amanda Foto,^a Amanda F. Ray,^a Alexander E. Nelms,^a Keyshawn L. Kennedy,^a Shelby G. Hartley,^a Logan M. Ryals,^a Chandan Gurung,^a William A. D'Angelo,^{a*}  Welkin H. Pope,^b  Dmitri V. Mavrodi^a

^aDepartment of Cell and Molecular Biology, School of Biological, Environmental, and Earth Sciences, The University of Southern Mississippi, Hattiesburg, Mississippi, USA

^bDepartment of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

ABSTRACT Here, we report the genome sequence of LuckyBarnes, a newly isolated singleton siphovirus that infects *Brevibacterium iodinum* ATCC 15728 and has a 50,774-bp genome with 67 predicted genes.

Actinobacteria encompass saprophytes, commensals, and pathogens, as well as producers of anticancer, anthelmintic, and antifungal secondary metabolites (1). Bacteriophages play a crucial role in the evolution of *Actinobacteria* and provide insights into the genetics and physiology of this economically important group of bacteria. Despite the apparent importance of actinobacteriophages, our understanding of their biology is limited to viruses of *Mycobacterium*, *Gordonia*, and *Arthrobacter*, while the knowledge of phages that infect other members of *Actinobacteria* is lagging (2). We report here the characterization of a new bacteriophage, LuckyBarnes, which was isolated from soil collected in D'Iberville, MS, using enrichment with *Brevibacterium iodinum* ATCC 15728 in a peptone yeast calcium (PYCa) medium. Genome analysis identified LuckyBarnes as a singleton siphovirus and as one of the only two *Brevibacterium* phages with sequenced genomes in the Actinobacteriophage database (3).

The bacteriophage was recovered by passing the enrichment culture through a 0.22- μ m filter and incubating the filtrate with *B. iodinum* at room temperature for 48 h, which resulted in 1-mm clear plaques. Transmission electron microscopy of LuckyBarnes revealed a 50-nm capsid and a 250-nm-long flexible tail (Fig. 1). Phage DNA was isolated using the Wizard DNA cleanup kit (Promega) and used to prepare a library with a NEBNext Ultra II FS kit (New England BioLabs), which was then sequenced on an Illumina MiSeq instrument with MiSeq v3 chemistry. The sequence run generated 1.42 million 150-bp single-end reads that were assembled in Newbler v.2.9 (4), with default parameters, to give a 50,774-bp contig with 3,954-fold coverage and a G+C content of 61.9%. No defined genomic termini could be identified, and to preserve gene contiguity, the genome start point was selected 2.74 kb upstream of the predicted terminase gene.

The genome sequence was analyzed with GeneMark v.3.25 (5), GLIMMER (6), tRNAscan-SE v.2.0 (7), and ARAGORN v.1.2.38 (8), followed by manual annotation using DNA Master v.5.0.2 (<http://cobamide2.bio.pitt.edu/computer.htm>), Starterator (<https://seaphages.org/software/>), and HHpred (9). Phamerator (10) analysis of the 67 predicted protein-coding genes revealed that most of them (86%) lack homologs in other actinobacteriophages. Several structural genes were similar to their counterparts from the *Arthrobacter* phage TripleJ (<https://phagesdb.org/phages/TripleJ/>). The genes account for a 94.7% coding capacity of the genome and are arranged into two genome arms transcribed in divergent directions, which is similar to cluster A mycobacteriophages (11). The 29.6-kb left genome arm contains virion structure and assembly genes, followed by genes for a terminase, a portal protein, a tail terminator, a tape measure protein, a major tail subunit, minor tail proteins, and lytic enzymes represented by a class II holin and an *N*-acetylmuramoyl-L-alanine amidase. Genes within the 20.6-kb

Citation Underwood SL, Foto A, Ray AF, Nelms AE, Kennedy KL, Hartley SG, Ryals LM, Gurung C, D'Angelo WA, Pope WH, Mavrodi DV. 2019. Discovery and characterization of bacteriophage LuckyBarnes. *Microbiol Resour Announc* 8:e00330-19. <https://doi.org/10.1128/MRA.00330-19>.

Editor John J. Dennehy, Queens College

Copyright © 2019 Underwood 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 Dmitri V. Mavrodi, dmitri.mavrodi@usm.edu.

* Present address: William A. D'Angelo, McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.

Received 27 April 2019

Accepted 30 May 2019

Published 20 June 2019

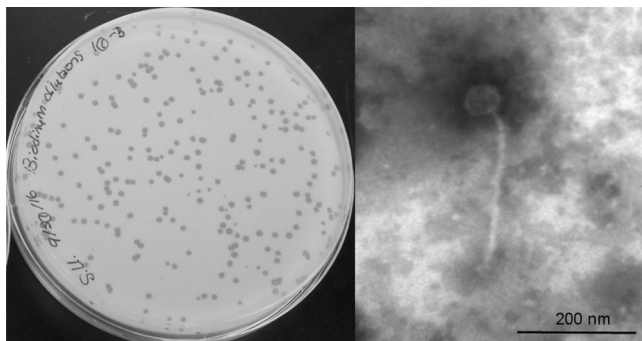


FIG 1 Plaque appearance (left) and virion morphology (right) of bacteriophage LuckyBarnes. For electron microscopy, high-titer lysate was applied on Formvar-coated grids, negatively stained with 1% phosphotungstic acid, and imaged with a Zeiss EM-109 transmission electron microscope (Carl Zeiss AG).

right genome arm encode several DNA metabolism enzymes, including a DNA polymerase, a primase, a DNA helicase, a single-stranded (ssDNA) binding protein, as well as a predicted dCMP-hydroxymethylase and two glycosyltransferases that may modify its DNA and improve resistance to degradation by host nucleases. The rest of the predicted genes (42%) encode conserved hypothetical proteins. Clear plaques and the absence of an integrase gene suggest that LuckyBarnes is a lytic phage.

Data availability. The genome of bacteriophage LuckyBarnes was deposited in DDBJ/ENA/GenBank under the accession number [MF668275](https://doi.org/10.1128/MF668275). The raw reads are available in the SRA under accession number [SRR8782908](https://doi.org/10.1128/SRR8782908).

ACKNOWLEDGMENTS

We thank Daniel A. Russell and Rebecca A. Garlena for conducting genome sequencing, quality control, and assembly at the University of Pittsburgh.

We acknowledge support from the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant P20GM103476. This project was also supported by The University of Southern Mississippi and the Howard Hughes Medical Institute Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science program (www.seaphages.org).

REFERENCES

1. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Meier-Kolthoff JP, Klenk H-P, Clément C, Ouhdouch Y, van Wezel GP. 2016. Taxonomy, physiology, and natural products of Actinobacteria. *Microbiol Mol Biol Rev* 80:1–43. <https://doi.org/10.1128/MMBR.00019-15>.
2. Hatfull GF. 2018. Mycobacteriophages. *Microbiol Spectr* 6:GPP3-0026-2018. <https://doi.org/10.1128/microbiolspec.GPP3-0026-2018>.
3. Russell DA, Hatfull GF. 2017. PhagesDB: the actinobacteriophage database. *Bioinformatics* 33:784–786. <https://doi.org/10.1093/bioinformatics/btw711>.
4. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen Y-J, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Ho CH, Irzyk GP, Jando SC, Alenquer MLI, Jarvie TP, Jirage KB, Kim J-B, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, et al. 2005. Genome sequencing in microfabricated high-density picoliter reactors. *Nature* 437:376–380. <https://doi.org/10.1038/nature03959>.
5. 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>.
6. Kelley DR, Liu B, Delcher AL, Pop M, Salzberg SL. 2012. Gene prediction with Glimmer for metagenomic sequences augmented by classification and clustering. *Nucleic Acids Res* 40:e9. <https://doi.org/10.1093/nar/gkr1067>.
7. Lowe TM, Chan PP. 2016. tRNAscan-SE on-line: search and contextual analysis of transfer RNA genes. *Nucleic Acids Res* 44:W54–W57. <https://doi.org/10.1093/nar/gkw413>.
8. 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>.
9. 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>.
10. 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>.
11. Hatfull GF. 2012. The secret life of mycobacteriophages. *Adv Virus Res* 82:179–288. <https://doi.org/10.1016/B978-0-12-394621-8.00015-7>.