




# Characterization and Genome Sequence of *Arthrobacter* Phage Iter

Kathleen A. Daly,<sup>a</sup> Audrey E. Nesbit,<sup>a</sup> Jacob D. Buchholz,<sup>a</sup>  Alison E. Kanak<sup>a</sup>

<sup>a</sup>Department of Biology, University of North Georgia, Dahlonega, Georgia, USA

**ABSTRACT** *Arthrobacter* phage Iter was isolated in North Georgia. Its genome is 43,963 bp with 70 open reading frames (ORFs) and a GC content of 67.4%. It shares 89.11% nucleotide identity with *Arthrobacter* phage Phives. Actinobacteriophages that share over 50% nucleotide identity are sorted into clusters, with Iter in cluster AZ.

There is growing concern about untreatable infections resulting from antibiotic-resistant bacteria that could be addressed through the use of phage therapy. The discovery and characterization of these viruses are important basic science that can be used to address clinical needs. Here, we present the *Arthrobacter* phage Iter, isolated using a Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) protocol (1).

Iter was isolated from soil collected in Georgia (34.470714°N, 83.975651°W) in 2022. *Arthrobacter globiformis* B-2979 was the host used for Iter isolation. Briefly, 7H9 liquid medium was added to soil and incubated at 37°C for 24 h. The sample was filtered using a 0.22- $\mu$ m filter. Phage presence was confirmed and purified via plaque assay and amplified to a high titer to extract phage genomic DNA. Electron microscopy of Iter identified it as a *Siphoviridae* with a capsid measuring 49.03 nm in diameter and tail measuring 123.74 nm long (Fig. 1). Iter formed plaques 4 mm in diameter with distinct margins featuring a “bull’s-eye” characteristic. Actinobacteriophages that share over 50% nucleotide identity are sorted into clusters and then further into subclusters where appropriate (2, 3). Iter was classified into cluster AZ.

Phage genomic DNA was extracted from Iter lysates with a Wizard DNA extraction kit (Promega) per the manufacturer’s instructions. A NEBNext Ultra II FS kit with dual-indexed barcoding was used to assemble a sequencing library. The library was run on an Illumina MiSeq system. There were 219,616 single-end 150-bp reads from the Iter library that provided 713-fold coverage of the Iter genome. These raw reads were assembled using Newbler v2.9 with default settings. The resulting single-phage contig was checked for completeness, accuracy, and phage genomic termini by using Consed v29 (4). The genome is 43,963 bp with a GC content of 67.4%.

The genome was annotated using GeneMark v3.25 (5), NCBI BLAST v2.9.0 (6), Glimmer v3.02 (7), HHpred v3.2.0 (8), ARAGORN v1.2.38 (9), and Phamerator (<https://phamerator.org>). Default parameters were used for all software unless otherwise specified. Hits with E values of 10e-10 or less were considered acceptable. Phamerator and GeneMark indicate that Iter has 70 open reading frames (ORFs), and 36 have predicted functions. Genes 1 to 37, 39 to 49, and 51 to 69 are transcribed in the forward direction, while genes 38, 50, and 70 are transcribed in the reverse direction. Iter is predicted to be a temperate phage, as a serine integrase (ORF51) was identified. A phamily was determined using Phamerator by using “pairwise comparisons to generate gene relationships” (10). As observed in other phamily members, Iter is predicted to code for a Cas4 family exonuclease (ORF26), which may play a role in nucleotide metabolism or CRISPR activity (11). Iter has 3’ sticky ends, with an 11-bp overhang. ORF14 and ORF15 encode tail chaperone proteins, which overlap a –1 frameshift predicted in ORF15 at nucleotide 10407, frequently observed in bacteriophages across many clusters. Iter is

**Editor** Catherine Putonti, Loyola University Chicago

**Copyright** © 2022 Daly 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/).

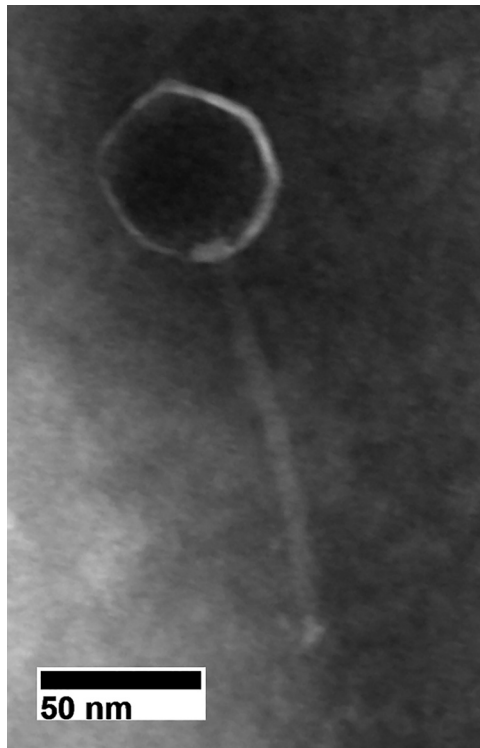
Address correspondence to Alison E. Kanak, [aekanak@ung.edu](mailto:aekanak@ung.edu).

The authors declare no conflict of interest.

**Received** 27 June 2022

**Accepted** 23 August 2022

**Published** 6 September 2022



**FIG 1** Transmission electron micrograph of Iter. TEM images were obtained using a JEM-1011 TEM (JEOL, Inc., Tokyo, Japan) at the University of Georgia Electron Microscopy facility.

genetically most similar to Phives (GenBank accession no. [MT889376](#)), with 89.11% nucleotide identity via BLAST alignment.

**Data availability.** Information on Iter's genome can be found in GenBank under accession no. [ON208833](#). Sequencing reads are part of the Sequence Read Archive with accession no. [SRX15121735](#) under BioProject accession no. [PRJNA488469](#).

#### ACKNOWLEDGMENTS

This research was conducted as part of the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program (3), supported by the Howard Hughes Medical Institute (HHMI). Funding was provided in part by the UNG College of Science and Mathematics professional development grant.

We thank John Shields and Mary Ard at UGA Georgia Electron Microscopy for transmission electron microscopy (TEM) imaging support. We thank Taylor Berry and Christina Miller for guidance during the genome preparation and annotation. We thank Sarah E. Miller, Alex J. Williams, Caroline M. Lantis, and Emily C. Mattison for their assistance in the genomic annotation of Arthrobacteriophage Iter. We finally thank Ryan Shanks and Miriam Segura-Totten for their assistance with manuscript editing and preparation.

#### REFERENCES

- 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](https://doi.org/10.1007/978-1-4939-7343-9_9).
- Hanauer DJ, Graham MJ, Betancur L, Bobrownicki A, Cresawn SG, Garlena RA, Jacobs-Sera D, Kaufmann N, Pope WH, Russell DA, Jacobs WR, Sivanathan V, Asai DJ, Hatfull GF, SEA-PHAGES. 2017. An inclusive Research Education Community (iREC): impact of the SEA-PHAGES program on research outcomes and student learning. *Proc Natl Acad Sci U S A* 114:13531–13536. <https://doi.org/10.1073/pnas.1718188115>.
- 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 Evolutionary 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>.
- Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. *J Comput Biol* 7:203–214. <https://doi.org/10.1089/10665270050081478>.
- 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>.
- Soding 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>.

7. 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>.
8. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R, Schwede T. 2018. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res* 46:W296–W303. <https://doi.org/10.1093/nar/gky427>.
9. Bienert S, Waterhouse A, de Beer TA, Tauriello G, Studer G, Bordoli L, Schwede T. 2017. The SWISS-MODEL Repository—new features and functionality. *Nucleic Acids Res* 45:D313–D319. <https://doi.org/10.1093/nar/gkw1132>.
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. Dixit B, Anand V, Hussain MS, Kumar M. 2021. The CRISPR-associated Cas4 protein from *Leptospira interrogans* demonstrate versatile nuclease activity. *Curr Res Microb Sci* 2:100040. <https://doi.org/10.1016/j.crmicr.2021.100040>.