| Bacteriophages | Announcement

Genome sequence and annotation of *Arthrobacter globiformis* phage Ruchi (AS1) isolated from soil in Lumpkin County, Georgia

Brooke Tatum,[1] Payton Murray,[1] Claire Bicknell,[1] Shane A. Webb,[1] Alison Kanak[1]

AUTHOR AFFILIATION See affiliation list on p. 2.

ABSTRACT Ruchi, a temperate, AS1 subcluster bacteriophage isolated in Lumpkin County, Georgia using host *Arthrobacter globiformis*, possesses a genome of 38,571 bp and 67.7% GC. Annotation of this virus revealed 64 predicted reading frames, no predicted tRNA genes, and a close evolutionary relationship to AS1 phage Basilisk.

KEYWORDS bacteriophage, genome annotation, arthrobacter phage, AS1

Phage therapy presents an alternative approach to the treatment of multidrug-resistant bacterial infections (1–3). A thorough understanding of phage diversity and evolution is therefore paramount. Here, we contribute to this knowledge with the annotated genome of Ruchi, a temperate AS1 subcluster bacteriophage propagated on *Arthrobacter globiformis*.

Ruchi was isolated in August 2022 from topsoil from the Pine Valley Recreation Center in Lumpkin County, Georgia (34.51N, 84.06W). Lab methods followed the SEA-PHAGES protocol (4). LB medium was mixed with soil and incubated at 30°C for 1 hour. Supernatant was then sterilized using 0.22 µm filtration. Phage presence was confirmed and purified by standard plaque assay using *A. globiformis* B-2979 and amplified to high titer via flooding of a web plate. A Wizard DNA extraction kit (Promega) was used on 6.7e−9 pfu/mL lysate to obtain 122.2 ng/µL genomic DNA. A NEBNext Ultra II FS kit was used for sequencing library construction. Illumina MiSeq sequencing yielded ~3,914× coverage from 1.1 million 150-bp single-end reads. Genome assembly used Newbler 2.9 (default settings), and the accuracy and completeness of the assembly were evaluated with Consed 29 (5). Ruchi has siphovirus morphology (Fig. 1) and is likely temperate based on plaque morphology and the presence of a tyrosine integrase gene.

DNA Master 5.23.6 (6), Glimmer 3.02 (7), GeneMark 3.26 (8), BLAST (9, 10), HHPred 2.08 (11), Phamerator 505 (12), tRNAscan SE 2.0 (13), Aragorn (14), and DeepTMHMM 1.0.24 (15) were used for genome annotation (all using default parameters). DNA Master provided first-pass analysis of open reading frames (ORFs), gaps, and ribosomal binding sites. Subsequent homology assessment using BLASTp (16) (Genbank nr database), HHPred (default pdb database; UniRef30), and Phamerator refined the annotation. Gaps larger than 25 bp were assessed for additional genes. An e value $<10^{-4}$ was used as the threshold for function assignments (17).

The complete Ruchi genome (38,571 bp; 67.7% GC; 3′ overhang GAGTTGCCGGGA) contains 64 predicted ORFs [36 with ascribed function (56%)] and no predicted tRNA genes. Genes 1–24 and 35–64 are encoded on one strand, and genes 25–34 are encoded on the other. Among the predicted genes are four nucleases, endolysin, an immunity repressor (adjacent to tyrosine integrase), and an excise protein as well as RusA-like resolvase. ORFs 14 and 15 are predicted to encode overlapping tail assembly chaperones (111 and 244 aa, respectively) with ORF 14 terminated by a −1 frameshift at nucleotide position 10336. Three predicted ORFs with assigned functions (ORFs 4, 16, 24) and five with unassigned functions (ORFs 1, 21, 22, 39, 47) likely have transmembrane domains.
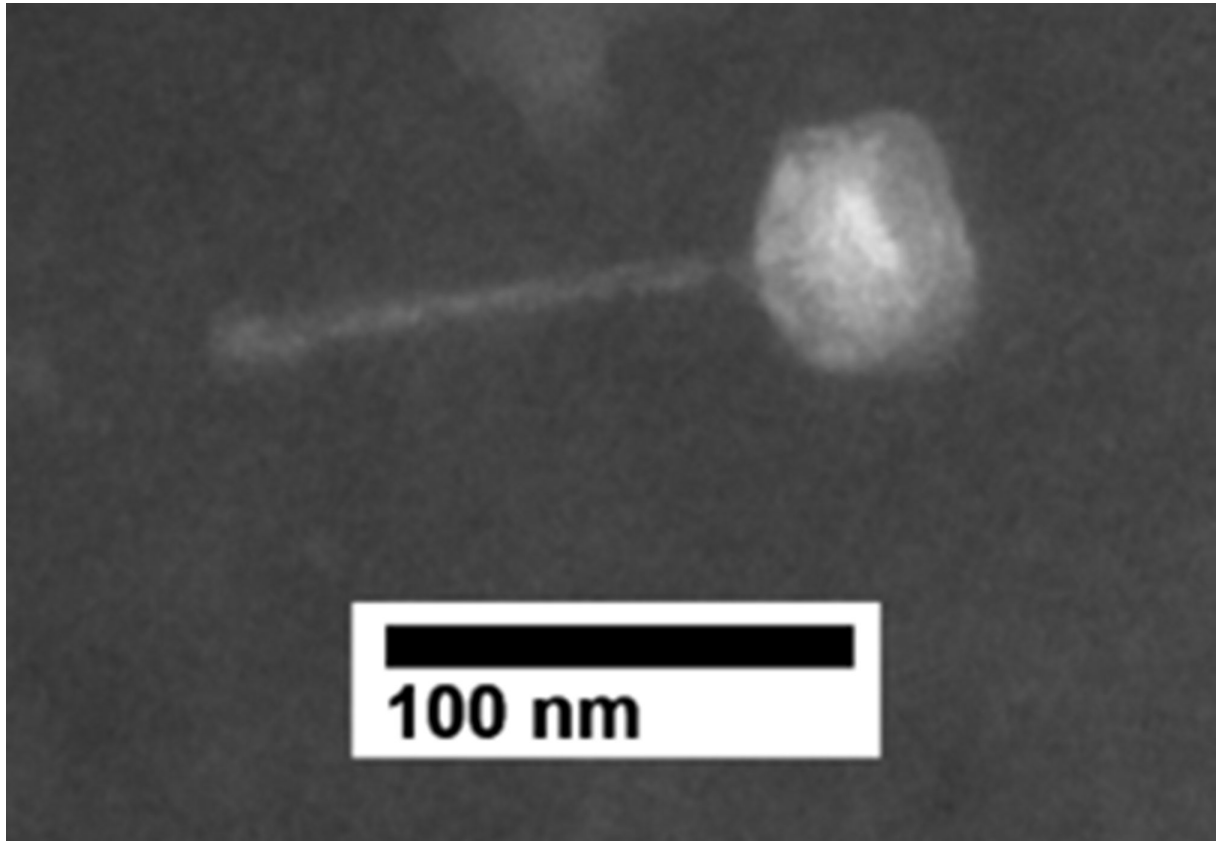
FIG 1 TEM image of phage Ruchi. Lysate was stained using the drop method with phosphotungstic acid. The image was obtained using a JEM-1011 TEM (JOEL, Inc., Tokyo, Japan) at the University of Georgia Electron Microscope Laboratory. Head diameter is ca 44 nm, and tail length is ca 117 nm.

Ruchi shares the highest nucleotide sequence similarity (98.7% identity) with *Arthrobacter* phage Basilisk (Genbank ON260822), which was isolated from Lumpkin County, GA, a year earlier about 20 km from Ruchi's locale.

AUTHOR AFFILIATION

[1]Department of Biology, University of North Georgia, Dahlonega, Georgia, USA

AUTHOR ORCIDs

Shane A. Webb ⓘ http://orcid.org/0009-0006-5669-404X
Alison Kanak ⓘ http://orcid.org/0000-0001-9053-250X

AUTHOR CONTRIBUTIONS

Brooke Tatum, Data curation, Formal analysis | Payton Murray, Data curation, Formal analysis | Claire Bicknell, Data curation, Formal analysis | Shane A. Webb, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Project administration, Validation, Writing – original draft | Alison Kanak, Conceptualization, Data curation, Investigation, Methodology, Supervision

DATA AVAILABILITY

The Ruchi genome can be accessed through NCBI (Genbank OR434022) and sequencing reads can be obtained from the SRA (SRX22366555).

REFERENCES

1. Jones JD, Varghese D, Pabary R, Langley RJ. 2022. The potential of bacteriophage therapy in the treatment of paediatric respiratory infections. Paediatr Respir Rev 44:70–77. https://doi.org/10.1016/j.prrv.2022.02.001
2. Marongiu L, Burkard M, Lauer UM, Hoelzle LE, Venturelli S. 2022. Reassessment of historical clinical trials supports the effectiveness of phage therapy. Clin Microbiol Rev 35:e0006222. https://doi.org/10.1128/cmr.00062-22
3. Vandamme EJ, Mortelmans K. 2019. A century of bacteriophage research and applications: impacts on biotechnology, health, ecology and the economy!: a century of bacteriophage research and applications. J Chem Tech Biotech 94:323–342. https://doi.org/10.1002/jctb.5810
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. 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
6. Lawrence J. 2021. DNA master version 5.23.6 (Build 2705; 24 Oct 2021)
7. 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
8. Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. implications for finding sequence motifs in regulatory regions. Nucleic Acids Res 29:2607–2618. https://doi.org/10.1093/nar/29.12.2607
9. National Center for Biotechnology Information (NCBI) [Internet]. 2023. Bethesda (MD) National library of medicine (US), National center for biotechnology information [1988]. Available from: https://www.ncbi.nlm.nih.gov/
10. 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
11. 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
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. Chan PP, Lin BY, Mak AJ, Lowe TM. 2021. tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. Nucleic Acids Res 49:9077–9096. https://doi.org/10.1093/nar/gkab688
14. 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
15. 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. Bioinformatics. https://doi.org/10.1101/2022.04.08.487609
16. Gish W, States DJ. 1993. Identification of protein coding regions by database similarity search. Nat Genet 3:266–272. https://doi.org/10.1038/ng0393-266
17. Colston LE, Segura-Totten M, Shanks RA. 2020. Characterization and genome sequence of the mycobacteriophage Donny. Microbiol Resour Announc 9:e00373-20. https://doi.org/10.1128/MRA.00373-20