microPublication BIOLOGY

3/28/2025 - *Open Access*

Genome Sequence of *Arthrobacter globiformis* B-2979 Phage Raphaella

Hannah Alapati[1], Adam Parks[1], Tyler Hildebrand[1], Joshua Leazer[1], Kateryn Rodriguez[1], John Patton[1§]

[1]Natural and Applied Sciences, Evangel University, Springfield, Missouri, United States

[§]To whom correspondence should be addressed: PattonJ@evangel.edu

Abstract

Bacteriophage Raphaella was isolated from a soil sample collected in Springfield, MO using *Arthrobacter globiformis* B2979-SEA. Raphaella has a genome of 51692 base pairs with a GC content of 62.6%, 96 putative protein encoding genes and one tRNA. It has been placed in the AY cluster of Actinobacteriophages based on gene content similarity.

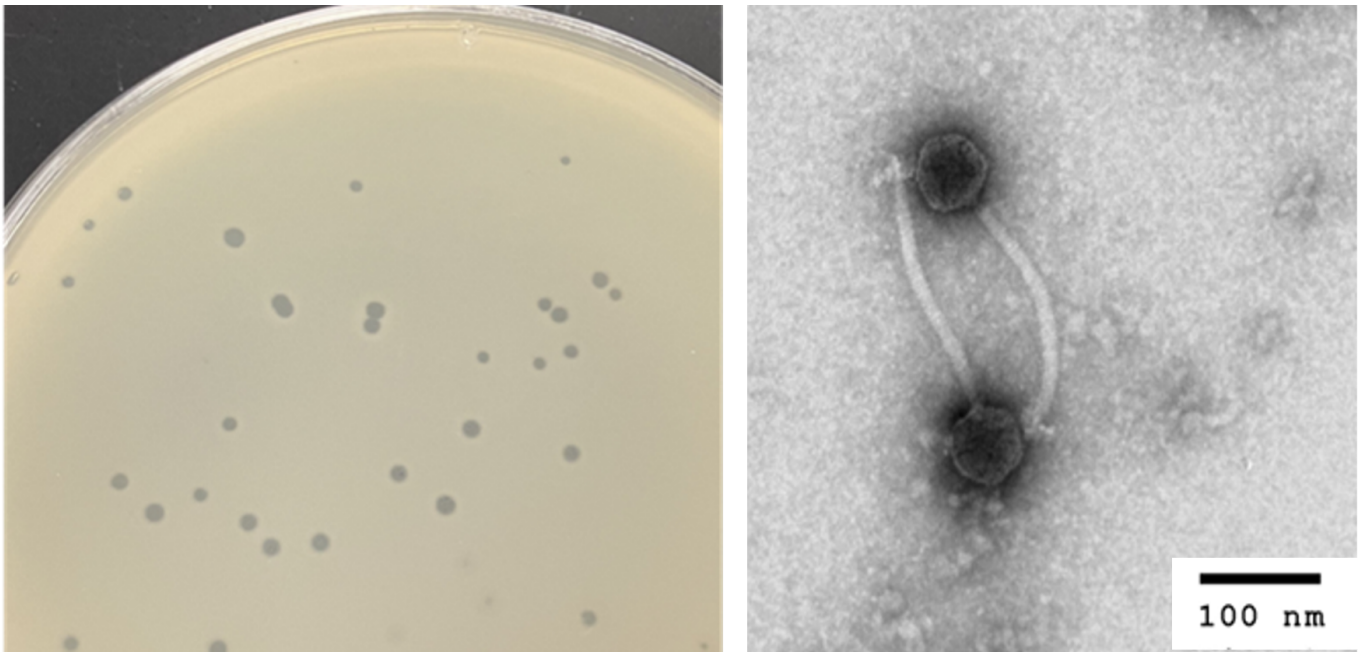

Figure 1. Plaque and virion morphology:

Left: Raphaella generates clear plaques with well-defined borders. Right: Raphaella particles have siphovirus morphology, with a polyhedral capsid and long, flexible tail.

Description

As antibiotic-resistant bacterial infections continue to rise, bacteriophages are being developed as an alternative therapeutic (Sulakvelidze et al., 2001, Strathdee and Patterson, 2019). In support of this development, the discovery and genetic characterization of novel phages is invaluable. Here, we report on the novel phage, Raphaella, which was isolated in September 2023, from a soil sample collected at Valley Water Mill Park in Springfield, MO (GPS: 37.26409 N, 93.24684 W). The soil sample was wet and contained small roots. This sample was suspended in peptone-yeast calcium (PYCa) liquid media, and the suspension was then centrifuged (2,000 x g, 10 min). The supernatant was filtered (0.2 micron pore size) before the filtrate was inoculated with *Arthrobacter globiformis* B2979-SEA. Following 3 days of incubation at 30˚C with shaking, an aliquot of the resulting culture was filtered. The filtrate plated in top agar with *A. globiformis*, giving rise to plaques of Raphaella after incubation of plates at 30˚C for 3 days. Raphaella was purified through three rounds of plating (Zorawik et al., 2024). Raphaella forms clear plaque with a diameter of 0.8 +/- 0.24 mm(n=5) (Zorawik et al., 2024) (Figure 1). A lysate was prepared and used for imaging virion particles by transmission electron microscopy using negative staining (1% uranyl acetate), revealing a capsid 59.2 +/- 1.9 nm (n=5) wide and the length of the tail was 210.7 +/- 15.8 nm (n=5) (Figure 1).

DNA of Raphaella was extracted from the lysate utilizing the Promega Wizard DNA clean-up kit, then sequenced on an Illumina MiSeq with v3 reagents after preparation with a NEB Ultra II Library Kit, which yielded 618710, 150 bp reads which constituted approximately 1701-fold coverage. These reads were assembled using Newbler v2.9 into a 51692 bp genome with

62.6% GC content, with 3' single-stranded genome termini determined using Consed v29 (Russell, 2018, Gordon and Green, 2013).

Raphaella's genome was automatically annotated using DNA Master v5.23.6 (Pope and Jacobs-Sera, 2018), embedded with Genemark v2.5 (Besemer and Borodovsky, 2005) and Glimmer v3.02 (Delcher et al., 2007). Translational starts were determined manually using the coding potential predicted in GeneMark (Besemer and Borodovsky, 2005) and then refined by comparison with similar genes using Starterator v578 (http://phages.wustl.edu/starterator/) and Phamerator v 578 (Cresawn et al., 2011). Putative functions were assigned to the genes using PECAAN (discover.kbrinsgd.org) and in the embedded BLASTp (Altschul et al., 1990) searches against the NCBI protein database and the actinobacteriophage database (Russell and Hatfull, 2016) as well as HHPred using PDB_mmCIF70, SCOPe70, Pfam-A, NCBI_Concerved_Domains (CD) databases (Söding et al., 2005). Utilizing ARAGORN v1.2.41 (Laslett and Canback, 2004) and tRNA scan v2.0 (Lowe and Eddy, 1997), a single tRNA which coded for the amino acid glycine was identified. Nine potential membrane proteins were found using DeepTMHMM v1.0 (Jeppe et al., 2022). All software were used with default setting. The annotation process revealed a total of 96 genes, 39 of which could be assigned putative functions. Based on gene content similarity of over 35% to phages in the Actinobacteriophage database, phagesDB, Raphaella was assigned to the AY cluster (Russell and Hatfull, 2016)

As with a majority of cluster AY phages, Raphaella encodes two tyrosine integrases. This, coupled with experimental evidence of lysogen formation by other cluster AY phages, suggests that Raphaella too is likely to establish lysogeny. We note, however, that no immunity repressor function could be predicted in Raphaella.

Nucleotide Sequence Accession numbers:

Raphaella is available at GenBank with Accession Number PP987873 and Sequence Read Archive Number SRX26311147.

Acknowledgements: We would like to thank Wandy Beatty at Washington University in St. Louis for her help in conducting TEM and producing wonderful images of Raphaella, Daniel Russell and Rebecca Garlena for their expertise in sequencing and assembling the genome, the SEA community for reviewing the manuscript, and the SEA-PHAGES program for support.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215(3): 403-10. PubMed ID: 2231712

Besemer J, Borodovsky M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Res 33(Web Server issue): W451-4. PubMed ID: 15980510

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. PubMed ID: 21991981

Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23(6): 673-9. PubMed ID: 17237039

Gordon D, Green P. 2013. Consed: a graphical editor for next-generation sequencing. Bioinformatics 29(22): 2936-7. PubMed ID: 23995391

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. DOI: 10.1101/2022.04.08.487609

Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32(1): 11-6. PubMed ID: 14704338

Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25(5): 955-64. PubMed ID: 9023104

Pope WH, Jacobs-Sera D. 2018. Annotation of Bacteriophage Genome Sequences Using DNA Master: An Overview. Methods Mol Biol 1681: 217-229. PubMed ID: 29134598

Russell DA, Hatfull GF. 2017. PhagesDB: the actinobacteriophage database. Bioinformatics 33(5): 784-786. PubMed ID: 28365761

Russell DA. 2018. Sequencing, Assembling, and Finishing Complete Bacteriophage Genomes. Methods Mol Biol 1681: 109-125. PubMed ID: 29134591

Söding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Res 33(Web Server issue): W244-8. PubMed ID: 15980461

Strathdee S, Patterson, T. (2019). The Perfect Predator: A Scientist's Race to Save Her Husband from a Superbug: a Memoir. Hachette Books.

Sulakvelidze A, Alavidze Z, Morris JG Jr. 2001. Bacteriophage therapy. Antimicrob Agents Chemother 45(3): 649-59. PubMed ID: 11181338

Zorawik M, Jacobs-Sera D, Freise AC, SEA-PHAGES, Reddi K. 2024. Isolation of Bacteriophages on Actinobacteria Hosts. Methods Mol Biol 2793: 273-298. PubMed ID: 38526736

Funding: This work was funded by the Department of Natural and Applied Sciences at Evangel University.

Author Contributions: Hannah Alapati: data curation, investigation, writing - original draft. Adam Parks: writing - original draft, investigation. Tyler Hildebrand: investigation. Joshua Leazer: investigation, writing - original draft. Kateryn Rodriguez: investigation. John Patton: investigation, data curation, project administration, writing - original draft, writing - review editing, supervision.

Reviewed By: Anonymous

History: Received December 15, 2024 **Revision Received** March 19, 2025 **Accepted** March 28, 2025 **Published Online** March 28, 2025 **Indexed** April 11, 2025

Citation: Alapati H, Parks A, Hildebrand T, Leazer J, Rodriguez K, Patton J. 2025. Genome Sequence of *Arthrobacter globiformis* B-2979 Phage Raphaella. microPublication Biology. 10.17912/micropub.biology.001464