Genome sequence of WestPM, a phage infecting *Microbacterium foliorum* isolated from beach environmental samples

Charles J. West^{1,2}, Brittany C. Yencho^{2,3}, Andrew J. Brown^{2,4}, Conor R. Flannigan^{2,5}, Hui-Min Chung^{2§}

¹Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL

²Department of Biology, University of West Florida, Pensacola, FL

³School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA

⁴United States Environmental Protection Agency, Gulf Breeze, FL

⁵Department of Surgery, Duke University, Durham, NC

[§]To whom correspondence should be addressed: hchung@uwf.edu

Abstract

Bacteriophage WestPM is a siphoviral-like phage infecting *Microbacterium foliorum* isolated from environmental samples collected on Pensacola Beach, FL. The genome of this phage is 39,693 bp long and contains 59 predicted protein-coding genes and zero tRNA genes. Based on gene content similarity, WestPM is grouped in the actinobacteriophage EA11 subcluster.

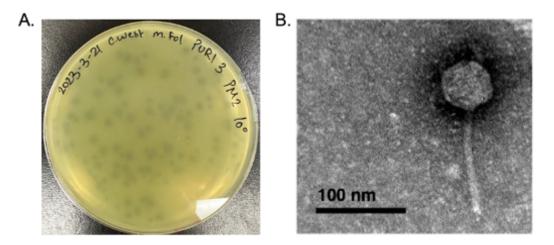


Figure 1. Morphological characterization of phage WestPM:

A. The plaque morphology of WestPM after three rounds of purification plated on PYCa medium. **B.** Negative staining transmission electron micrograph of *Microbacterium foliorum* phage WestPM revealing siphovirus morphology. Staining was performed using 1% uranyl acetate staining on Formvar-coated copper grids. Imaging was performed using a Hitachi H-7650 microscope operated at 75 kV and equipped with a 1kx1k CCD detection camera (Gatan 782). The magnification used was 200,000× with a pixel size of 5.18 Å at the level of the specimen. WestPM has a capsid with a diameter between 53-54 nm and a flexible tail of 110-118 nm (n = 2).

Description

Bacteriophages are the most abundant biological entities on Earth and play critical roles in ecological balance, nutrient cycling, and microbial evolution (Chevallereau et al., 2021; Mayers et al., 2023). Here we report a *Microbacterium foliorum* phage, WestPM, which was isolated from a mixture of coquina and surface sand collected from the shore of Pensacola Beach, Florida (GPS coordinates: 30.33073 N, 87.14191 W). For direct isolation, the sample was suspended in 14 mL PYCa (peptone-yeast extract-calcium) liquid medium for two days, and the supernatant was then filtered (0.22 µM). Viral particles in the filtrate were precipitated with PEG solution (PEG 1000), and the precipitate was then resuspended in 0.14 mL buffer before being plated in top agar with *Microbacterium foliorum* NRRL B-24224 (Russell et al., 2019). After three days at 28°C, a representative ~1.5 mm-wide hazy plaque with a halo and irregular border was isolated, yielding WestPM (**Figure 1A**). WestPM was purified through three rounds of plating, and negative-staining transmission electron microscopy (TEM) revealed siphovirus morphology (Bradley, 1962; Zorawik et al., 2024) (**Figure 1B**).

1/4/2025 - Open Access

DNA from WestPM was extracted from a phage lysate using the Norgen Biotek phage DNA isolation kit, prepared for Illumina sequencing using the NEB Ultra II Library Kit, and sequenced using an Illumina MiSeq (v3 reagents), generating 1,730,886 single-end 150-base reads, constituting 6541-fold coverage. After contig assembly through Newbler v2.9 and a check for completeness and genome termini through Consed v29, WestPM was determined to have a circularly permuted genome of 39,693 bp and possessing 63.9% GC content (Silva et al., 2013; Gordon et al., 1998; Russell and Hatfull, 2016). The genome was annotated using DNA Master v5.23.6 (Pope and Jacobs-Sera, 2017), PECAAN version 20230302 (Rinehart et al., 2016), Glimmer 3.02b (Delcher et al., 2007), GeneMark v2.5 (Besemer and Borodovsky, 2005), BLAST using Actinobacteriophage and NCBI non-redundant protein databases (Sayers et al., 2021), HHpred using PDB_mmCIF70, Pfam-A_v37, and NCBI Conserved Domains databases (Söding et al., 2005), DeepTMHMM 1.0.24 (Hallgren et al., 2022), and Phamerator (Cresawn et al., 2011), all using default parameters.

The annotation revealed 59 predicted genes, and 28 were assigned putative functions. Aragorn and tRNAscan-SE revealed the lack of tRNA genes within the genome (Laslett and Canback, 2004; Chan and Lowe, 2019). Genes within the first half encode structural and assembly functions and are transcribed rightward, whereas the DNA metabolism functions and 16 genes of unknown function are encoded in the second half of the genome and are transcribed leftward. Based on gene content similarity (GCS) of at least 35% to phages in the Actinobacteriophage database, phagesDB (https://phagesdb.org), WestPM was assigned to phage subcluster EA11 (Gordon et al., 1998; Russell and Hatfull, 2016), despite WestPM being isolated from beach sand and coquina samples while all other EA11 phages were isolated from soil samples. Notably, WestPM genes *38*, *49*, and *50* do not have homologs in other EA11 phages.

Data Availability

The sequence information of WestPM is available in GenBank with the accession number <u>PP978895</u>, and Sequence Read Archive (SRA) No.<u>SRX24338412</u>.

Acknowledgements: We would like to thank Daniel Russell and Rebecca Garlena for assembling the genome, the SEA-PHAGES program for providing support on genome annotation and lysate archiving, Che-Yu Cheng and Dr. Nai-Chun Lin from the Department of Agricultural Chemistry at National Taiwan University for performing electronic imaging.

References

Besemer J, Borodovsky M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Research 33: W451-W454. DOI: <u>10.1093/nar/gki487</u>

Bradley DE. 1962. A Study of the Negative Staining Process. Journal of General Microbiology 29: 503-516. DOI: <u>10.1099/00221287-29-3-503</u>

Chan PP, Lowe TM. 2019. tRNAscan-SE: Searching for tRNA Genes in Genomic Sequences. Methods in Molecular Biology, Gene Prediction : 1-14. DOI: <u>10.1007/978-1-4939-9173-0 1</u>

Chevallereau A, Pons BtJ, van Houte S, Westra ER. 2021. Interactions between bacterial and phage communities in natural environments. Nature Reviews Microbiology 20: 49-62. DOI: <u>10.1038/s41579-021-00602-y</u>

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*(1), 395. DOI: <u>10.1186/1471-2105-12-395</u>

Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23: 673-679. DOI: <u>10.1093/bioinformatics/btm009</u>

Gordon D, Abajian C, Green P. 1998. *Consed:* A Graphical Tool for Sequence Finishing. Genome Research 8: 195-202. DOI: <u>10.1101/gr.8.3.195</u>

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. : 10.1101/2022.04.08.487609. DOI: <u>10.1101/2022.04.08.487609</u>

Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res. *32*(1), 11–16. DOI: <u>10.1093/nar/gkh152</u>

Mayers KMJ, Kuhlisch C, Basso JTR, Saltvedt MR, Buchan A, Sandaa RA. 2023. Grazing on Marine Viruses and Its Biogeochemical Implications. mBio 14: 10.1128/mbio.01921-21. DOI: <u>10.1128/mbio.01921-21</u>

Pope WH, Jacobs-Sera D. 2017. Annotation of Bacteriophage Genome Sequences Using DNA Master: An Overview. Methods in Molecular Biology, Bacteriophages : 217-229. DOI: <u>10.1007/978-1-4939-7343-9_16</u>

1/4/2025 - Open Access

Rinehart CA, Gaffney B, Wood JD, Smith J. 2016 PECAAN, a Phage Evidence Collection and Annotation Network. https://discover.kbrinsgd.org/login

Russell DA, Garlena RA, Hatfull GF. 2019. Complete Genome Sequence of Microbacterium foliorum NRRL B-24224, a Host for Bacteriophage Discovery. Microbiology Resource Announcements 8: 10.1128/mra.01467-18. DOI: <u>10.1128/MRA.01467-18</u>

Russell DA, Hatfull GF. 2016. PhagesDB: the actinobacteriophage database. Bioinformatics 33: 784-786. DOI: <u>10.1093/bioinformatics/btw711</u>

Sayers EW, Bolton EE, Brister JR, Canese K, Chan J, Comeau DC, et al., Sherry. 2021. Database resources of the national center for biotechnology information. Nucleic Acids Research 50: D20-D26. DOI: <u>10.1093/nar/gkab1112</u>

Silva GG, Dutilh BE, Matthews TD, Elkins K, Schmieder R, Dinsdale EA, Edwards RA. 2013. Combining de novo and reference-guided assembly with scaffold_builder. Source Code for Biology and Medicine 8: 10.1186/1751-0473-8-23. DOI: 10.1186/1751-0473-8-23

Soding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Research 33: W244-W248. DOI: <u>10.1093/nar/gki408</u>

Zorawik M, Jacobs-Sera D, Freise AC, SEA-PHAGES, Reddi K. 2024. Isolation of Bacteriophages on Actinobacteria Hosts. Methods in Molecular Biology, Phage Engineering and Analysis : 273-298. DOI: <u>10.1007/978-1-0716-3798-2_17</u>

Funding: The Office of Undergraduate Research at UWF provided undergraduate research awards and traveling awards to Andrew Brown, Conor Flannigan, Charles West, and Brittany Yencho. Hui-Min Chung was supported by the NSF grant 1711842.

Author Contributions: Charles J. West: data curation, formal analysis, methodology, writing - original draft, writing - review editing, validation, visualization. Brittany C. Yencho: data curation. Andrew J. Brown: data curation. Conor R. Flannigan: data curation. Hui-Min Chung: data curation, conceptualization, writing - original draft, writing - review editing, investigation, funding acquisition.

Reviewed By: Anonymous, Kristen Butela

History: Received October 23, 2024 Revision Received December 18, 2024 Accepted January 2, 2025 Published Online January 4, 2025 Indexed January 18, 2025

Copyright: © 2025 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: West, CJ; Yencho, BC; Brown, AJ; Flannigan, CR; Chung, HM (2025). Genome sequence of WestPM, a phage infecting *Microbacterium foliorum* isolated from beach environmental samples. microPublication Biology. <u>10.17912/micropub.biology.001395</u>