

SUPPLEMENTAL 1

A. Transmission Electronic Microscopy Methodology

TEM of phage 113 was performed by adsorbing phages to glow-discharged carbon-coated 400-mesh copper grids for three minutes and then staining them with 2% uranyl acetate in water, rinsing briefly, and then air-drying. The grids were visualized on an FEI Tecnai 12 TEM at 80 kV. Images were captured on a Gatan Rio 16 CCD camera with GMS 3 software.

B. Phage 113 Comparative Genome Analysis Methodology

An enterococcal phage proteomic tree was constructed by submitting the assembled phage 113 FASTA sequence to ViPTree (1). ViPTree utilizes normalized tBLASTx scores between viral genomes to calculate genomic distance for phylogenetic proteomic tree analysis. Phages 113 and EFDG1 were compared using the genome alignment view in ViPTree. Regions of similarity between the genomes were detected by tBLASTx and color-coded to reflect the percentage of protein sequence identity. Phage genome nucleotide identity was determined by submitting the annotated phage 113 genome to the Phage Galaxy Comparative Genomics workflow version 2021.01 (2). Genome nucleotide identity matrix generated from progressive mauve was imported into GraphPad Prism v9.1.1 to generate the heat map (3).

C. Data Availability of Accession Numbers

The Illumina DNA sequencing reads have been deposited in the National Center for Biotechnology Information under the following accession number: SAMN19047785 (phage 113) and the European Nucleotide Archive under the following accession number: PRJEB39873 (phage 9184). The assembled bacteriophage genomes were submitted to GenBank and were assigned the following accession numbers: MZ147816 (phage 113), MT939240 (phage 9181), MT939241 (phage 9183) and MT939242 (phage 9184).

REFERENCES

- 1) Nishimura Y, Yoshida T, Kuronishi M, Uehara H, Ogata H, Goto S. 2017. ViPTree: The viral proteomic tree server. *Bioinformatics* 33:2379-2380.
- 2) Ramsey J, Rasche H, Maughmer C, Criscione A, Mijalis E, Liu M, Hu JC, Young R, Gill JJ. 2020. Galaxy and Apollo as a biologist-friendly interface for high-quality cooperative phage genome annotation. *PLoS Comput Biol* 16:e1008214.
- 3) Darling AE, Mau B, Perna NT. 2010. progressiveMauve: Multiple genome alignment with gene gain, loss and rearrangement. *PLOS One* 5:e11147.