CONSIDER FOR TALK

2024 SEA Symposium Abstract

LeTourneau University

Longview TX

Corresponding Faculty Member: Fred Baliraine (fredbaliraine@letu.edu)



Diamond Love

Isolation and Characterization of Mycobacterium phage FreddyB, the First Cluster F Phage Isolated in East Texas

Diamond Love, Matilda S McDaniel, Sherlyn A Diaz, Josh D McLoud, Frederick N Baliraine

Bacteriophages (phages) are important in pathogen identification and phage therapy. Using the enriched method with Middlebrook 7H9 medium and *Mycobacterium smegmatis* mc2 155 as the host, *Mycobacterium* phage FreddyB was isolated from a soil sample collected at LeTourneau University in Longview, TX (32.463333 N, 94.7275 W), on August 22, 2023. Phage presence was confirmed using the spot test. Phage purification was done through three rounds of ten-fold serial dilutions and plating on Middlebrook 7H9 top agar, followed by webbed-plate preparation and flooding with phage buffer. This yielded a high titer lysate of 3.2 x 1010 PFU/ml. Phage FreddyB produced small, turbid plaques (average diameter 1.1 mm, range 0.7 – 2.2 mm; n = 10) after 48 h of incubation at 37°C. Negative-stain TEM showed FreddyB to be a siphovirus with an isometric capsid measuring ~43 nm (range 41 - 45 nm; n = 5) in diameter and a long, flexible, non-contractile tail measuring ~156 nm (range 137 - 178 nm; n = 5) in length. Following DNA extraction using the Promega Wizard® DNA Clean-Up Kit, Illumina genome sequencing was done at the University of Pittsburg. Annotation was done using DNA Master, Glimmer, GeneMark, Starterator, PhagesDB, NCBI, HHPred, Phamerator, Aragorn, tRNAscan-SE and DeepTMHMM. FreddyB has a 10 bp 3’ sticky overhang (CGGACGGCGC), genome length 58318 bp, 61.7% GC content, no tRNA, and 113 putative genes. All, except 9 genes (8%), were transcribed in the forward direction. Notably, FreddyB had many (34) small genes (<200 bp), 10 of which were strikingly tiny (45-93 bp; gp 36, 38, 49, 51, 68, 85, 89, 90, 100). Several of these were not predicted by GenMark during autoannotation but have 4 bp overlaps with their flanking upstream and downstream genes. FreddyB is a temperate phage, whose lifecycle is evidenced by the presence of the tyrosine integrase (gp 43), immunity repressor (gp 46), the Cro (gp 47) which plays a key role in lysogenic to lytic state induction, and the excise gene (gp 50). Functions, including 5 membrane proteins, could be assigned to only 44.5 % (50/113) of the putative genes. FreddyB was assigned to sub-cluster F1, based on ≥35% gene content similarity (GCS) to other phages, using the GCS tool in PhagesDB, and it is the first F cluster phage isolated in East Texas.