DO NOT CONSIDER FOR TALK

2024 SEA Symposium Abstract

Metropolitan Community College

Omaha NE

Corresponding Faculty Member: Bhaswati Manish (bmanish@mccneb.edu)

The World of Novel Phages Welcomes FortCran, a Bacteriophage with a Large Genome to be Annotated

Josephine R McLean, Laura M LaMarche, Rolande Kanyala, Amy LaDue, Bhaswati Manish

Bacteriophages are viruses that “eat” bacteria cells and amplify through hijacking the replication cycle of bacteria. Metropolitan Community College (MCC) has partnered with HHMI (Howard Hughes Medical Institute) to run the SEA-PHAGES (Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science) for the first time, starting in the Fall of 2023. Through this program, **FortCran**, a novel phage that infects the bacteria *Gordonia rubripertincta*, was discovered by Amy Ladue and Josephine McLean, students at MCC. Discovery of FortCran was initiated by collecting soil sample on a warm (88°F) night on October 1st, 2023, 5-7 inches deep in the soil layers under pine trees off the side of the interstate by Amy Ladue at 7:00pm. This soil sample was treated to both direct and enriched isolation attempts. Both the direct and enriched samples were used to perform plaque assays, and spot tests using serial dilutions. However, only the enriched sample resulted in plaques and warranted further tests in the hopes of discovering a novel phage. After isolating the plaques, it was adopted by Josephine McLean who purified it through three rounds of purification.

This allowed us to see FortCran’s plaque morphology; FortCran has clear plaques with clear boundaries that vary somewhat in size. Next, Josephine amplified the phage lysate to a titer of 4.1 x 10^9pfu by webbed plate technique and extracted the phage DNA. Phage DNA yield measured by nanodrop was found to be 250.3 ng/µL. Further, the DNA quality was characterized via restriction enzyme digestion using *HaeIII*, *MseI* and *SacII* and performing 0.8% gel electrophoresis. The phage lysate was subjected to Electron Microscope (EM) imaging for visualizing phage morphology. EM imaging was performed for this phage at UNMC (University of Nebraska Medical Center), and UMBC (University of Maryland, Baltimore County) showed that FortCran has a capsid diameter of about 70nm and a tail length of about 250nm. DNA sequencing using Illumina technology performed at the University of Pittsburgh revealed that FortCran is a lytic phage of the DJ cluster.

This phage genome contains 59,848 base pairs (phagesdb.org), GC content is 51.5%, and has three reverse genes. In the synteny related to FortCran, the closest relative appears to be another DJ cluster phage, Artorias. This genome is currently being annotated by students enrolled in MCC’s SEA-PHAGES Bioinformatics program using PECAAN, DNAMaster, BLAST, Staterator, Phamerator, GeneMarkS, and Glimmer.