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2024 SEA Symposium Abstract

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CapTrips: Saga of discovering a putatively benevolent phage against the soil bacteria, Gordonia rubripertincta

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Exploring new bacteriophages/phages (viruses that eat bacteria) can grant us a greater understanding of the genomics of soil bacterial communities. Using phages, researchers can discover unique characteristics of the bacteria itself and contribute significantly to the fields of biomedicine and agriculture.

In the Fall of 2023, Metropolitan Community College (MCC) in collaboration with Howard Hughes Medical Institute (HHMI) brought the HHMI’s phage discovery and genomics program, Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) to its campus. As part of the Phage Discovery component, soil sample was collected by Sean Mathews from a creek bed at Hickory Hills Park, located in the suburbs of Omaha. Direct and enriched isolation procedures were both conducted with the sample. After obtaining a positive result with the enriched isolate, as evidenced by appearance of plaques on the PyCa agar plates for the plaque assays, a well separated plaque was picked from the plate. Further rounds of purifications were performed by utilizing the techniques of plaque assay, spot titer tests, and serial dilution. Next, the purified plaque was amplified to obtain the phage titer of 5X10^9. The phage DNA was extracted and quantitated by Nanodrop spectrophotometer to obtain a DNA yield of 170.2 ng/µl. DNA characterization was performed by agarose gel electrophoresis of HaeIII, MseI, and SacII restriction enzyme digested samples. Finally, the DNA sample was sequenced at the Hatfull laboratory in the University of Pittsburgh using Illumina sequencing. Electron microscope (EM) imaging was performed with the collected high titer phage lysate at UMBC Baltimore’s EM core facility that helped to view the phage morphology. This entire procedure enabled the discovery of a unique phage, which was named **CapTrips** by its discoverer Sean Mathews. Information on CapTrips is available at the Actinobacteriophage database phagesDB.org.

EM imaging captured images revealed CapTrips morphology to be that of a Siphoviridae with a capsid size of ~60nm and tail length of ~280nm. DNA sequencing results showed CapTrips belongs to DJ cluster of Actinobacteriophages with a genome size of 59,629bp containing 3' Sticky Overhang (CGCCGCTCT), 9 bases long.
Currently the CapTrips genome is undergoing annotation using arrays of Bioinformatics tools such as DNA Master, NCBI Blast, PECAAN, Phamerator maps, and their features such as GeneMarkS, Glimmer, Starterator, and HHPred. In the synteny related to CapTrips, the closest relative appears to be another DJ cluster lytic phage, Avazak.