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Isolation and Characterization of ChuckDuck, a Cluster FA phage

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Discovering and characterizing novel bacteriophage is necessary for advancing phage therapy and expanding knowledge of viral diversity and abundance. In collaboration with the SEA-PHAGES project at the University of Pittsburgh, students at Stevenson University were able to isolate novel bacteriophages that infect the soil-dwelling bacterium, *Arthrobacter globiformis B-2979*. The class collected multiple soil samples from different areas on and off camp and used enriched isolation to increase the chances of finding phage. Spot tests were used to identify soil samples that contained phage. At least one sample from each of the eleven lab groups in the class had phage that could infect the host bacteria. In total, 56% of samples were positive for phage. Each group then selected one sample and performed three rounds of 10-fold serial dilutions to purify the samples. Purified samples showed consistent plaque morphologies. To increase the DNA titer for sequencing and archiving in the SEA- PHAGES database, the samples were amplified by creating webbed plates. Using transmission electron microscopy (TEM) at the University of Maryland, Baltimore County, all eleven groups successfully imaged their phage. One of the eleven phages, ChuckDuck, which had a titer of 6.6 x 10^9 pfu/ml and DNA concentration of 62.7 ng/uL was sent to the University of Pittsburgh for Illumina sequencing. This phage had small, uniform, circular, non-turbid plaques with clear borders. The TEM image of ChuckDuck indicated it was a siphovirus with a capsid diameter of 54.5 nm and tail of 169.7 nm in length. Chuck Duck's genome was auto-annotated with DNA Master version 5.23.6 Build 2705, GeneMark version 2.5p, and Glimmer. ChuckDuck is currently assigned to cluster FA and has been characterized as a temperate virus, despite having non-turbid plaques. The sequence annotation is currently being manually curated and is predicted to have a total sequence length of 42,833 bp and contain 68 genes, including 2 potential tRNA genes. Both Aragorn and tRNAScan predicted 1 tRNA gene at position 41995-42072 in the genome. tRNAScan also predicted another tRNA gene with a low Infernal score of 25.1 at position 33615-33702. Further inspection of these possible tRNA genes will be conducted and validated. One predicted gene has no homology to other known phams and is being further investigated. This semester, we will continue to annotate ChuckDuck to add its genome to the SEA-PHAGES database.