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Characterization of Eight New Actinobacteriophages from Charleston, South Carolina

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In Spring 2018, 44 students in the College of Charleston Molecular Biology labs collected environmental samples and attempted to isolate bacteriophages that attack the host bacterium Mycobacterium smegmatis. Using direct isolation or enrichment techniques, members of the class obtained high titer lysates for eight new bacteriophages. After genomic DNA was extracted from each of these viruses, restriction analysis and PCR-based cluster analysis were performed to better characterize each. Students also examined the morphology of these actinobacteriophages by transmission electron microscopy and data collected for each virus was entered in the Actinobacteriophage Database at PhagesDB.org.
The eight new bacteriophages isolated include: Awendaw, Carmen, Cashua, Guwop3, Hacker94, Lolalove, Luna22, and Revamp. Based on the preliminary PCR analysis of four bacteriophages tested, the class hypothesized that Awendaw, Cashua, and Hacker94 are all members of the A1 cluster and that Luna22 is not a member of the clusters evaluated (A1, A2, B1, B2, B3, C1, C2, D, E, F, H1, H2, or I). The class also determined which of six restriction enzymes (BamHI, ClaI, EcoRI, HaeIII, HindIII, or SalI) cut each actinobacteriophage genome. Genomes were cut by the indicated restriction enzymes: Awendaw and Cashua (only HaeIII), Carmen (ClaI, EcoRI, HaeIII, SalI), Guwop3 (None cut; may need to retest), Hacker94 (ClaI, HaeIII), Lolalove (None cut; ClaI and HaeIII untested), Luna22 (BamHI, ClaI, HaeIII, SalI), Revamp (BamHI; ClaI, EcoRI and HaeIII untested). Finally, a comparison of the bacteriophage morphologies is also presented. Based on their appearances and the restriction analysis results, we hypothesize that all eight bacteriophages are distinct members of the Siphoviridae. Each of the viruses has been prepared for archiving and will be submitted to the University of Pittsburgh in Fall 2018.