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Isolation and Characterization of the Novel Gordonia Bacteriophage Survivors

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In the Fall of 2022, one section of introductory biology students at Indiana University Southeast isolated 9 novel bacteriophages: Aloki, MadCow, NachoLibre, NoName, Oogie, Spiders, Survivors, Vordorf, and Winnieston. These phages were enriched and isolated using the bacterial host *Gordonia rubripertincta*, a bacterium commonly found in soil. Transmission electron micrograph (TEM) images were collected for this set of 9 bacteriophages and high-titer lysates were prepared. Of this set of nine, seven were ultimately submitted for archiving at the University of Pittsburgh and inclusion in the Actinobacteriophage Database. DNA extraction from this set of high-titer lysates was unexpectedly challenging. Ultimately, DNA was prepared from two phages (Vordorf and Survivors), using PCI (phenol, chloroform, isoamyl alcohol) extraction. Only one of these two DNA samples proved to be suitable for genome sequencing, the aptly named phage Survivors. Genome sequencing and Phamerator processing revealed that phage Survivors is a member of cluster CT, with a genome length of 45,436 base pairs. Auto-annotation of the genome identified 68 features. Manual annotation is currently underway, conducted by one section of second-semester introductory biology students. Eight of these students continued from the Phage Discovery course, and seven students began their phage research in the Phage Genomics semester. Manual annotation is being conducted using programs such as DNA Master, BLAST, Genemark, Phamerator, and HHPred. Some highlights of specific gene functions identified so far include a tape measure protein, lysins A and B, and a DNA helicase protein, plus at least one orpham.