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2025 SEA Symposium Abstract

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Genomic Analysis of B1 Subcluster Mycobacteriophages Mesmerelda and ElvisPhasley (and a Rogue Cupriavidus Phage)

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The discovery and characterization of novel mycobacteriophages not only provide key resources to address the growing problem of antibiotic resistance, but also serves as a promising source of new molecular tools for engineering mycobacteria. In Fall, 2024, each William & Mary SEA-PHAGES student collected and isolated a bacteriophage infecting Mycobacterium smegmatis. Phage lysates were purified, and students obtained a TEM photograph, isolated DNA, and ran gel electrophoresis analysis. Four phages, ElvisPhasley, Mesmerelda, Raid, and Neighley, were sent to the University of Pittsburgh for sequencing. Two of the phages, ElvisPhasley and Mesmerelda, are both part of the 290-member B1 subcluster (as of March 2025) with respective lengths of 69,502 and 68,890 base pairs. Both are lytic phages, as evidenced by clear plaques and the absence of integrase proteins. Mesmerelda and ElvisPhasley share 97.8% nucleotide identity with approximately 90% of their phams conserved. Of note, both phages encode helix-turn-helix DNA binding proteins, but they belong to different phams. Annotation revealed 103 and 101 total genes for Mesmerelda and ElvisPhasley, respectively, many of which had unknown functions. TEM imaging shows a siphoviral morphotype for both phages. In addition to M. smegmatis, M. aichiense was also used as a host. However, due to likely contamination from a soil sample, this resulted in the discovery of a novel Cupriavidus gilardii phage which made for an interesting teaching moment and entailed intriguing bioinformatic detective work.