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9th Annual SEA-PHAGES Symposium Abstract

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Isolation, Characterization, and Genomic Analysis of Mycobacteriophages ActinUp (K1) and Boyle (B2)

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Mycobacteriophages ActinUp (K1) and Boyle (B2) were isolated from soil samples collected on the campus of Seton Hill University in Greensburg, PA and characterized by first-year undergraduate students participating in a one-semester combined phage discovery and bioinformatics SEA-PHAGES research course. Both phages were obtained through enrichment isolation at 25°C using the bacterial host *Mycobacterium smegmatis* mc2155, with ActinUp producing turbid plaques and Boyle producing clear plaques after 48 h incubation at 28°C, indicating potential temperate and virulent properties, respectively. The genomes of ActinUp (59.8 kb, 66.6% GC, defined linear ends) and Boyle (67.5 kb, 69.0% GC, circularly permuted) were annotated using PECAAN, DNA Master, HHPred, Phamerator, Starterator, tRNAScan-SE, Aragorn, and the Blast program suite. ActinUp contains 96 putative protein-coding genes and 1 tRNA-Trp. ActinUp is highly similar (≥95% average nucleotide identity) to previously characterized Cluster K1 phages, with the organization of the *attP* site and start associated sequences determined to be similar to other K1 phages. ActinUp gp91 and gp92 are currently under investigation for similarity to HicAB, a horizontally mobile RNA-targeting toxin-antitoxin cassette previously identified in numerous bacterial and archaeal genomes. Boyle contains 92 protein-coding genes and no tRNAs and is highly similar (≥95% average nucleotide identity) to other Cluster B2 phages. Like other Cluster B2 phages, Boyle contains a putative gene coding for lysin A (gp47) but not lysin B, and several other genes in Boyle yield BlastP matches to genes identified in *Mycobacterium abscessus* prophages.