CONSIDER FOR TALK

8th Annual SEA-PHAGES Symposium Abstract

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Bioinformatics discoveries brought to life: uncovering mycobacteriophage gene functions

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The SEA-PHAGES program at Lehigh University is a collaborative undergraduate research enterprise focused on isolating and characterizing phages that infect Actinobacter hosts to gain a better understanding of phage genome structure, gene function, and phage biology in general. In addition to uncovering new phages for comparative genome analysis, our program has focused on characterizing phage gene products that function in lytic infection to evaluate their potential as biocontrol agents to target pathogenic bacteria. Here we report on the genomic characterization of four of our most recently discovered novel phages (Derek [C1], Annyong [A4], James [F1], and Taptic [W]) that infect *Mycobacterium smegmatis*. We also present an update on the functional characterization of one of several phage gene products encoded by orphams found in the N cluster phage, *Mycobacterium* phage Butters. Of particular interest among our newly discovered phages is *Mycobacterium* phage Taptic, a third member of the W cluster. With a genome size of 60,973 bp, Taptic has a 91 ORFs, all positioned on the forward strand – an organization common in genomes of only a few mycobacteriophage clusters. Taptic plaques are miniscule (<1mm) and clear - the latter suggestive of a lytic phage. Annotation of a putative function for gp38 as a helix-turn-helix repressor (>99% probability in HHPred) for transcriptional regulation suggests several possibilities, including a putative lysogenic cycle for Taptic. To explore this possibility, lysogeny experiments were performed. The presence of Taptic mesas and positive patch tests were suggestive of Taptic lysogens; however, stable lysogens were not formed as host cells were consistently lysed over time. The role of gp38 in the Taptic life cycle remains to be explored. In other experiments, the computational prediction that Butters gp31 is a transmembrane protein was tested by expressing a C terminal tetracysteine-tagged gp31 and control ORFs within *E. coli* and imaging tagged proteins using fluorescence microscopy. Data show that Butters gp31 resides within the *E. coli* membrane coincident with a membrane marker. Further analysis of Butters gp31 function as a membrane protein is underway to determine how this membrane protein may function in a mycobacterial host. In summary, we will present a comparative analysis of four novel mycobacteriophage genomes and provide an update on functional studies of a newly characterized membrane protein encoded by Butters gp31. Overall, our bioinformatics efforts continue to highlight genomic features of interest for laboratory exploration.