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8th Annual SEA-PHAGES Symposium Abstract

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The Isolation and Genomic Analysis of Mycobacterium smegmatis and Rhodobacter capsulatus bacteriophages

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The SEA-PHAGES program was established to expand the knowledge of bacteriophages by giving young adults the opportunity to isolate and analyze their own phage. Through this program in the past two semesters, Illinois Wesleyan's SEA-PHAGES class isolated sixteen phages that infect *Mycobacterium smegmatis* MC2 155 (*Ms*) and six that infect *Rhodobacter capsulatus* strain YW1 (*Rc*). *Ms* is a species in the phylum Actinobacteria which is commonly found in moist soils while *Rc* is a photosynthetic member of the phylum Alphaproteobacteria that is typically found in aqueous environments. *Rc* has been used frequently as a model system in studying photosynthesis and horizontal gene transfer via the phage-like particle known as Gene Transfer Agent, but has rarely been used in bacteriophage studies. Whereas the extensively-studied *Ms* phages now total over 1,000 isolates, only six phages that infect *Rc* had been isolated before our work. This made studying *Rc* phages fascinating, but also difficult because there was little knowledge concerning them. After isolation, our phages were phenotypically characterized and categorized according to their plaque morphology, ability to form lysogens, susceptibility of these lysogens to infection by other phages, and, for the *Rc* phages, their ability to infect related host strains. Based on these analyses, three small-plaque-forming phages that were unable to form lysogens were chosen for genomic sequencing from the *Ms* host while three phages with diverse plaque morphologies and host ranges were chosen for the *Rc* host. The genomes for each of the *Ms* phages, Yucca, Erdmann, and Daffodil, were found to be relatively large (~155 Kbp), encode many tRNAs, and were highly similar to each other. Based on these characteristics and their relatedness to other known phages (including two found by the 2011 IWU SEA-PHAGES class), each were classified as members of the C1 cluster. The genomes for our three *Rc* phages, Dormio, McDreamy, and Tiptonus, were found to be much more diverse in size, 41.6 Kbp, 68.2 Kbp, and 95.5 Kbp, respectively, and notably unique. While Dormio was found to be similar to a previously known *Rc* phage singleton, both McDreamy and Tiptonus have genomes that are much larger than the average known *Rc* phage genome size of 39.1 Kbp and do not cluster with any of these known *Rc* phages. This information thus allowed us to form a new cluster “Rc-C” and add two new singletons to the growing family of *Rc* phages. Lastly, an examination of our Rc-C phage genomes allowed us to come up with two hypotheses to account for our observed differences in host range between these two highly similar members of this new cluster. In summary, our results indicate that our utilization of a well-studied host (*Ms*) and a novel host (*Rc*), was successful in allowing us to better understand the diversity of phages and will also hopefully provide a stepping-stone for future pursuers of the same goal.