CONSIDER FOR TALK

8th Annual SEA-PHAGES Symposium Abstract

Western Carolina University

Cullowhee NC

Corresponding Faculty Member: Maria Gainey (mdgainey@email.wcu.edu)

Preliminary Cluster Typing of 20 Novel Bacteriophages Discovered at Western Carolina University

Reliza McGinnis, Erin Cafferty, Megan Eckardt, Jamie R Wallen

Western Carolina University joined the SEA-PHAGES program as part of cohort 8 in 2015. During our first year of phage hunting 20 unique mycobacteriophages were successfully isolated by students. Three bacteriophages named Adahisdi, TipsytheTrex and Serendipitous were selected by the class for full genome sequencing and annotation. These bacteriophages were found to belong to clusters A1, A2, and B5, respectively. Interestingly B5 is a rare cluster with only 5 other genomes sequenced. TipsytheTrex on the other hand showed great similarity to the prototypic mycobacteriophage L5. The diversity of the bacteriophages that were chosen for sequencing prompted us to determine the overall cluster diversity of the bacteriophages discovered by our class this year. To achieve this goal we combined four different methods that allowed us to putatively assign almost all phages discovered to a cluster or subcluster. The first method involved lysogen immunity testing. We created lysogens from subcluster A1, A2, and E bacteriophages. These lysogens were first tested for homoimmunity to their parental bacteriophages, and were then challenged by all bacteriophages discovered by the class to test repressor compatibility. For the second method we examined restriction enzyme digest results from the class and inputted the number of cut sites into the Phage Enzyme Tool 2.0 maintained by faculty at the University of Louisiana at Monroe. The Phage Enzyme Tool allowed us to determine a list of potential clusters that our newly isolated bacteriophages may belong to. We then performed a tapemeasure gene PCR with cluster or subcluster specific primers as described by Smith et al. 2013. In addition to these other methods we also performed shot gun cloning using SalI digestion and were able to sequence an approximately 500bp section of 13 of the 20 bacteriophages discovered by the class. These tests allowed us to look at different aspects of each virus and attempt to make a cluster assignment without full genome sequencing. While these methods are not 100% accurate, taken together these results have given us a nice window into the cluster diversity of the bacteriophages discovered at Western Carolina University this year. Most bacteriophages belonged to cluster A, however we have also likely discovered 4 F1, and one cluster E bacteriophage. In the future we plan to expand our bacteriophage discovery to nearby Great Smokey Mountains National Park to begin to get an idea of mycobacteriophage diversity in our region.