CONSIDER FOR TALK

10th Annual SEA Symposium Abstract

Alliance University (formerly Nyack College)

New York NY

Corresponding Faculty Member: Jackie Washington (washjd4@gmail.com)



Sucely Ponce Reyes



Christal Rolling

Non-Mycobacterial Actinobacteriophages Expanding Our Knowledge of Phage Biology and Evolution

Sucely Ponce Reyes, Christal Rolling, Mariana Pereira Moraes, Maridalia Lillis, Maria I Paschalis, Grace K Anger, Marissa K Antonucci, Christina Dukehart, Rachel M Ewers, Kaelan Kanai, Franklin Mercado, Chelsea D Nichols, Ralph L Nicholson, Sofia M Osorio, Xylvie X Santiago, Djulie S Scaff, Kryscell N Stoner, Kathryn G Tamondong, Makayla Veracka, Daniel Kaluka, Peter J Park, Jacqueline M Washington

Compared to mycobacteriophages, much less is known about other actinobacteriophages. Consequently, isolation and characterization of non-mycobacterial phages will increase the diversity of bacteriophages and increase our knowledge about phage biology and evolution. During 2017-2018, Nyack College Phage Hunters isolated phages using several different hosts, including *Gordonia terrae* CAG3, *Gordonia terrae* 3612, *Rhodococcus erythropolis* NRRL B-1574 , and *Microbacterium foliorum* NRRL B-24224. Twenty phages were isolated, seven of which were sequenced. They include the *Gordonia* phages, EmsquaredA (CY1), TillyBobJoe (DC), Maridalia (CZ1), Easley (CZ4) and Nedarya (A15); *Rhodococcus* phage Shuman (CA) and *Microbacterium* phage Paschalis (EC). This diverse collection of sequenced phages range in size from 46,544-58,677bp.   
  
As not much is known about many of these new non-mycobacterial actinobacteriophages, identification of gene functions can be a challenge. Gene order is mostly conserved in phages with the structural genes on the left end of the genomes. On this end, phages have both a small and large terminase gene used to package the genome in the empty capsid. Whereas, the large terminase subunit is more easily identified, the small terminase subunit is not. As it dimerizes, sequence analysis using the program COILS2 was used to try to identify the small terminase subunits. Bioinformatic analysis also revealed that Paschalis maybe atypical in regards to structural gene order. In most phages, the head to tail connector complex proteins are between the major capsid and major tail protein. In Paschalis, initial functional characterization places the head-to-tail connectors downstream the major tail protein. Paschalis also contains a unique gene, rifampin ADP-ribosyl transferase, which has been attributed to the resistance of *Mycobacterium smegmatis* to the antibiotic rifampin by glycosylation. To date it has not been identified in the genus *Microbacterium*.  
  
Temperate phages usually have either a serine or tyrosine integrase. Interestingly, TillyBobJoe has both a serine and tyrosine integrase which appears to be typical for cluster DC as all 16 current members of the cluster have two integrases. The tyrosine recombinase integration site is predicted to be at tRNA Thr in the bacterial chromosome. Consequently, to further investigate the predicted tyrosine recombinase integration site we designed primers to probe the attL and attP sites in order to determine the frequency of integration using the tyrosine integrase as well as the stability of the lysogens.  
  
All of our sequenced phages with the exception of Paschalis are predicted to be temperate and should form lysogens. Consequently, we have attempted to isolate lysogens from all our phages to perform immunity assays. Results show that some of these actinobacteriophages have a heterotypic defense mechanism similar to that found in mycobacteriophages.