CONSIDER FOR TALK

11th Annual SEA Symposium Abstract

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Investigating Gordonia terrae phages for the presence of prophage-mediated host defenses

Swathi Tata, 2018-2019 Pitt SEA-PHAGES Course, Meghan Bechman, Rebecca Bortz, Kristen Butela, Brenda Hammer, Marcie Warner, Matthew Montgomery, Deborah Jacobs-Sera, Graham F Hatfull

The collection of over 15,000 actinobacteriophages, with 2,900 of these being sequenced, can reveal insights into phage diversity and evolution. The 2018-2019 student cohort of phagehunters at the University of Pittsburgh isolated 198 phages from environmental samples using the host *Gordonia terrae* 3612, contributing to the overall total of over 1,500 phages isolated on *Gordonia* hosts. The genomes of 19 of these phages were sequenced, adding to the existing collection of 379 sequenced *Gordonia* phages. These phage genomes are characteristically mosaic and span considerable genomic diversity, being assigned to 8 extant clusters (CS [4], CT [1], CV [1], CZ [5], DB [1], DC [2], DE [4], and DP [1]). One sample yielded two fully sequenced phages (JajaA [Cluster CV] and JajaB [Cluster CS3]) that we purified, separated, assigned to cluster using PCR, and annotated. Additional phages previously isolated by Pitt students in years 2016-2018 were annotated and assigned to 5 clusters (CV [2], CZ [3], DB [1], DC [1], and DN [2]). Phages isolated during the 18-19 academic year differ in genome length ranging from 46,096 bp (HannahD, Cluster DB) to 114,220 bp (Boopy, Cluster DS), and G+C% content, ranging from 50.1% (Ziko, Cluster DP) to 67.6% (Bakery, Cluster DC; *G. terrae* is 67.8%). Several temperate *G. terrae* phages in our collection display genetic mosaicism in the area immediately surrounding the immunity cassette, similar to what has been previously reported for the Cluster N mycobacteriophages. Such genes are candidates for novel prophage-mediated viral defense mechanisms. To investigate this possibility, previous students in the Pitt SEA-PHAGES course created a collection of *G. terrae* lysogens using Blueberry (Cluster CV), Utz (Cluster CV), UmaThurman (Cluster CV), Lilas (Cluster CY1), Vasanti (Cluster CZ2), Adora (Cluster CZ4), and Opie (Cluster DB). We tested a collection of over 100 sequenced and unsequenced phages for their ability to infect these lysogens, and preliminary data shows that these lysogens defend against infection from various phages, with plating efficiencies reduced by at least 10-4 relative to the wild-type *G. terrae* host. Future directions include identifying candidate genes that could confer defense against superinfection in the lysogens we tested this year, generating lysogens from newly annotated temperate phages that show genetic mosaicism near the immunity cassette, sequencing the phages against which the various prophages tested here provide protection, and investigating lysogen gene expression and the phages they defend against.