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7th Annual SEA-PHAGES Symposium Abstract

Hope College

Holland MI

Corresponding Faculty Member: Aaron Best (best@hope.edu)

Lysis Cassette Mosaicism and Potential Expanded Host Range Evident in the Genomes of Glass and Bella96

Keagan Belyk, Jacob Conroy, Kyle Cushman, Morgan Dalman, Monica Elliot, Joseph Fifer, Haley Fischman, Ester Fletcher, Kevin Franz, Sean Gitter, Jessica Guillaume, Austin Gutting, Emma Hardy, Abagail Jeavons, Clara Jurik, Emily Kain, Danny Kosiba, Adam Krahn, Sieun Lee, Alyssa Machay, Emily Mejicano-Gormely, Kim Nguyen, Nicholas Parliament, Matthew Petrovich, Chase Platte, Hayley Reitsma, Alison Rich, Jada Royer, Maxwell Sievers, Stephen Talaga, Mary Clare Theis, Natalie Vela, Philip Versluis, Yong Chul Yoon, Molly Bogolin, Carl Deeg, Aaron Best, Joseph Stukey

Thirty-five new mycobacteriophages were isolated from soil samples collected around the state of Michigan and parts of the United States. All were capable of infecting *Mycobacterium smegmatis* at 32°C and produced a variety of plaque morphologies based on size, shape, and clarity. Both lytic and temperate phages appear represented in this collection. All phages were experimentally characterized with respect to temperature range (32°C and 42°C). Cluster specific patterns in the temperature conditions were observed; 12 of 13 predicted Cluster K isolates failed to infect *M. smegmatis* mc2155 at 42°C. Two mycobacteriophages, Glass and Bella96, were chosen for complete genome sequencing and comparative genomic analyses. The predominant plaque produced by Glass at 32°C was turbid and 0.5-1.0mm in diameter, while plaques produced at 42°C were clear and 1.0-1.5mm in diameter. The predominant plaque produced by Bella96 at after 5 days at 32°C was turbid and 3 mm in diameter, whereas no plaques grew at 42°C. Complete genome sequences for Glass and Bella96 revealed relationships to members of Clusters B2 and K1, respectively. The genome of Glass is 67.5 Kb, 69.0% GC, and contains 94 genes; the genome of Bella96 is 60.7 Kb, 66.1% GC, and contains 98 genes, including 1 tRNA(Trp-CCA) gene. Twenty-seven (29%) and thirty-two (33%) protein coding genes were assigned functions in Glass and Bella96, respectively, based on comparative analyses. An analysis of the lysis cassette (*lysA*, *lysB*, holin) reveals the mosaic nature of this region in both Clusters B2 and K1. Glass (B2) does not contain an identifiable *lysB*, consistent with other B2 phages. The arrangement of the lysis cassette in B2 phages is atypical, with *lysA*-holin genes in tandem in a forward orientation amidst a series of reverse genes. The distribution of the phams is broad for the *lysA* gene, but restricted to the B2 cluster and a single B4 phage for the putative holin gene. This pattern of pham distribution for the lysis cassette in Bella96 (K1) is similar – broad *lysA* distribution, restricted *lysB* distribution, and restricted holin distribution. The genome of Bella96 contains two novel ORFs, being found in several other draft K genomes isolated this year. Bella96 gp26 is found in the midst of well-conserved K1 minor tail proteins and replaces gp26 of other K1 phages that are part of a 40 member pham. The second ORF, Bella96 gp78, is found in a total of 8 K1 and K5 draft phages; comparative analyses suggest similarity to a membrane binding protein from *Segniliparus rugosus*, a bacterium that also contains mycolic acids in its cell wall structure. This hints at the possibility of host range expansion of some cluster K members beyond the genus *Mycobacterium*.