CONSIDER FOR TALK

11th Annual SEA Symposium Abstract

Hope College

Holland MI

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6° of Separation: The Impact of Temperature on Isolation of Cluster K Mycobacteriophages

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Mycobacteriophages are viruses that infect mycobacterial hosts. Over 1750 mycobacteriophages are organized into 39 distinct clusters based on genetic similarity. Some cluster A and K mycobacteriophages can also infect Mycobacterium tuberculosis, a distinction of potential medical importance. Hope College SEA-PHAGES students have been isolating possible cluster K mycobacteriophages at a higher frequency (≈ 2x) after lowering the isolation temperature from 37°C to about 32°C (30°-32°C). These 32°C-isolated phages were unable to propagate at 42°C. PCR analysis supported cluster K classification for many possible cluster K phages isolated at 32°C (23 of 30 tested of a total of 43), but few isolated at 37°C (3 out of a total of 23). All 3 PCR-supported K phages isolated at 37°C grew at 42°C. We have sequenced 16 of the possible cluster K phages, and found all PCR-supported phages, including the 3 isolated at 37°C, belong to cluster K. We hypothesize that the observed higher Cluster K phage isolation frequency is at least partly due to a relative growth advantage at lower temperatures that is fully compromised at 42°C. Results from experiments testing specific growth parameters, including phage thermostability, adsorption rate, latent period, and burst size, are consistent with our hypothesis. In an effort to determine the step of the lytic cycle blocked at 42°C, a temperature down-shift experiment (42°C shifted down to 32°C) was performed on Hyperbowlee, a cluster K phage isolated at 32°C and growth inhibited at 42°C. Assays were performed testing two different 42°C hold time lengths. Both assays produced a consistent shift in time to first release of new Hyperbowlee phage, to a point about 85 minutes following the temperature down-shift. These results and subsequent tests indicated that phage infection was blocked, post DNA transfer, at about 20-30 minutes into the 32°C lytic cycle. Additional investigations into phage stability under drying conditions and competition growth assays are underway.