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9th Annual SEA-PHAGES Symposium Abstract

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Effects of Temperature on the Isolation and Growth of Known and Predicted Cluster K Mycobacteriophages

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Mycobacteriophages are viruses that infect mycobacterial hosts. Over 1300 mycobacteriophages have been organized into at least 34 distinct groupings or clusters based on genomic sequence similarity. Some mycobacteriophages from Clusters A and K can also infect *Mycobacterium tuberculosis*, a distinction of potential medical importance. Recently, Hope College SEA-PHAGES students have been isolating predicted Cluster K phages at a higher frequency (≥ 2x) after changing the enrichment growth temperature from 37°C to 32°C. Additionally, many of these phages were unable to propagate at 42°C. PCR analysis supported a Cluster K classification for most predicted Cluster K phages isolated at 32°C (all temperature sensitive for growth at 42°C), but for only one of the phages (Ruthiejr) isolated at 37°C. Ruthiejr does propagate at 42°C. We initially hypothesized that Cluster K phages isolated at 32°C have a relative growth advantage at lower temperatures. Further, we were interested in identifying in those phages, the step(s) of the lytic growth cycle inhibited at the higher growth temperature. We investigated temperature-dependent growth properties including phage thermostability, adsorption rate, lytic growth cycle time (latent period), and burst size, of several known and PCR-supported Cluster K mycobacteriophages. We found the ability to propagate at 42°C was not always consistent with phage stability and/or host adsorption kinetics at 42°C. For example, phages Bella96 (K1), Krueger (K6), Polymorphads, and Hyperbowlee are all growth-defective at 42°C. However, whereas Bella96 and Krueger show reduced stability at 42°C, Polymorphads and Hyperbowlee were thermostable. Ruthiejr, the PCR-supported cluster K phage isolated at 37°C was also stable at 42°C. Interestingly, Ruthiejr and Hyperbowlee, despite their different growth temperature profiles, both showed very similar host adsorption kinetics at 42°C. This suggests that for Hyperbowlee, infection initiation, or the shift from free to irreversible bound phage, is also not affected at 42°C. Our initial one-step growth analyses of D29 (control), Bella96, and Krueger showed impaired growth of the two Cluster K phages at ≥ 37°C compared to D29. These results now suggest that some Cluster K phages may have a relative growth disadvantage at temperatures ≥ 32°C. Initial efforts to isolate phage-resistant *M. smegmatis* mutants have yielded several candidates showing both phage-specific and non-specific resistance phenotypes. Our findings provide insight into the growth behavior of Cluster K phages that may lead to discoveries about *M. smegmatis* and *M. tuberculosis* infection by mycobacteriophages.