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Investigation of B1 and F1 Subcluster Mycobacteriophages’ Life Cycle, Genomes, and Potential Applications for Detection of Pathogenic Mycobacteria

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Detection of pathogenic mycobacteria is emerging as a potential application of mycobacteriophages. Characterizing phage life cycle, phage-host interactions, and the phage genome is crucial in order to determine whether a phage will be a suitable candidate for diagnostic application. We studied phages from subclusters B1 and F1 to investigate these characteristics.

Phages AltPhacts, DaWorst, and Alexphander were isolated from soil using *M. smegmatis* as a host. AltPhacts presented both clear and turbid plaques, suggesting the possibility that AltPhacts was temperate. However, whole genome sequencing and PHACTS analysis classified AltPhacts as a lytic B1 phage. Other B1 phages have also been observed to produce mixed plaque morphologies, prompting an investigation of genomic elements that might produce this result. Genome comparison and BLASTp analyses within the B1 subcluster suggested DNA helicase variants may have been horizontally transferred from different bacterial species and may affect plaque morphology. Additionally, Phyre2 structural analysis identified gp46 as a lambda-like repressor DNA binding protein with 97.6% confidence. Phylogenetic analysis and multiple sequence alignment of gp46 from B1 phages with either clear or mixed plaques revealed divergent sequences due to a conserved two amino acid variation. In summary, genetic variants within the B1 subcluster may explain variation in plaque morphologies, and although AltPhacts is lytic, it may still have potential application as a detector or even therapeutic agent of *M. tuberculosis*.

In addition to *M. tuberculosis*, there are other serious pathogens of the *Mycobacterium* genus that require new diagnostic developments as well. To evaluate whether F1 subcluster phages like Alexphander and DaWorst could serve as diagnostic agents of pathogenic nontuberculous mycobacteria, phage replication time and potential infectivity of pathogenic *M. abscessus* were assessed. A one-step growth curve determined DaWorst’s latent period to be 100 minutes, around three hours shorter than most mycobacteriophages. Phylogenetic analysis demonstrated Alexphander’s Y-integrase gene to be similar to that of a *M. abscessus* subsp. bollettii prophage, suggesting that *M. abscessus* may be within Alexphander’s host range. Similar GC content and patterns of codon usage bias between Alexphander, DaWorst, and *M. abscessus* further suggested potential infectivity, although no conclusive results were found. These two F1 subcluster mycobacteriophages’ short latent period and potential infectivity of *M. abscessus* suggest they and other F1 mycobacteriophages may be suitable for use as reporter phage candidates for mycobacteria. For all three phages, further research to confirm host range and to explore the incorporation of reporter genes will determine whether they can contend as detection agents for pathogenic mycobacteria.