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Isolation and analysis of Gordonia terrae phages reveals novel aspects of lysis, virion morphology, and host range

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Analysis of more than 1,000 *Mycobacterium smegmatis* bacteriophage (mycobacteriophage) genomes provides significant insights into phage diversity and evolution. To gain a broader perspective on phage biology, University of Pittsburgh students utilized an alternate host, *Gordonia terrae* 3612, for phage isolation. *G. terrae* is a member of the same taxonomic order (Corynebacteriales) as *M. smegmatis*; this relatedness facilitates analysis of the evolutionary pressures affecting different classes of phage genes. Isolation of *Gordonia* phages may also have medical and industrial applications: *Gordonia* species are implicated in catheter-related infections in humans and as contributors to wastewater sludge. Pitt students isolated 182 *Gordonia* phages; 30 of which have been sequenced and annotated. These *Gordonia* phages have been categorized into 8 clusters and 12 singletons, and their diversity appears to be at least as great if not greater than the mycobacteriophages. The *Gordonia* phage genomes have an average length of 54,897 bp (ranging from 41,857 bp [Nyceirae] to 98,136 bp [Yvonnetastic]), average G+C content of 65.4% (ranging from 58.9% [Hotoboro] to 68.4% [Phinally]; the host G+C content is 67.8%), and 88 genes (ranging from 66 [Emalyn] to 198 [Yvonnetastic]). *Gordonia* phage genomes share similar architectures with mycobacteriophages: the left arms contain the virion structure and assembly genes, genes involved in lysogeny (if present) are centrally located, and the right arms contain DNA metabolism genes, orphams, and genes of no known function. Despite genome architecture similarities to mycobacteriophages, *Gordonia* phages display a number of distinct attributes. The enzymatic functions of mycobacteriophage lysin A’s are commonly separated into two polypeptides in *Gordonia* phages and lysin B genes are found in unexpected locations. Numerous phages isolated on *G. terrae* were also able to infect one or more of the following hosts: *G. lacunae*, *G. westfalica*, *G. rubripertincta*, and *G. neofelifaecis*. This promiscuity in host range is rarely observed in the mycobacteriophages. *Gordonia* phages also exhibit distinct morphological features. *Gordonia* phages frequently possess exceptionally long, flexible tails; tape measure genes in Hotorobo, Benczkowski14, and Katyusha range in size from 9,084 bp to 9,984 bp and are among the longest such genes discovered. To date, Pupper is the sole contractile tailed *Gordonia* phage to be identified. Furthermore, Cluster CZ (composed entirely of *Gordonia* phages) is the first cluster to contain phages with both prolate and isometric capsids; this can likely be attributed to the discovery of variability in the major capsid genes present in individual members of this cluster. These distinctive aspects of *Gordonia* phages, combined with the discovery of a large collection of new orphams in these phages, support the notion that the phage population is vast and continually expanding.