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Usefulness of Restriction Enzymes to Predict Cluster Assignments of Novel Gordonia Bacteriophages

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Restriction enzyme digestion patterns have been used successfully to predict the cluster assignment of unsequenced Mycobacterium smegmatis phages. The extension of this approach to phages isolated on the Gordonia terrae host has proven more difficult due to a higher degree of sequence diversity within existing Gordonia cluster designations and phage ability to elude the host’s restriction system. Recently, we submitted a mixture of DNA from ten Gordonia terrae isolates as a Dogems (Deconvolution of Genomes after En Masse Sequencing) sample. Each isolate’s DNA was digested with a panel of restriction enzymes prior to sequencing in an attempt to predict their cluster assignment. While the sequencing data provided unique contigs for each isolate, the virtual digestion of each contig with the NEB cutter virtual restriction enzyme tool did not provide definitive matches to any of the individual experimental digests. Direct Nanopore MinION sequencing of isolates has positively identified three samples (Derg, Dusty, and Eggsie), while prior restriction digestion has provisionally identified a fourth (Oofdah). In an effort to understand these results, we began analyzing the restriction enzymes used in an effort to account for the differences in the observed in situ versus in silico digestion patterns. We have concluded that phage DNA modification (e.g., methylation) is likely responsible for much of the observed discrepancies. We have begun an evaluation of several restriction enzymes, including AfIII, AvrII, KpnI, NcoI, NdeI, and PstI, that are reported to be unaffected by select methylation. In situ and in silco digests of phage DNA with these enzymes were compared to assess their utility in predicting cluster assignments. Preliminary results indicate that some of these enzymes may be more useful for cluster prediction than those used previously.