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Production of Polyclonal Antibodies Against Structural Proteins of Viruses from Different Clusters Accompanied by Annotation of the Viral Genomes

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Of 16 mycobacteriophages isolated in 2017, the genomes of three were sequenced and annotated: Sibs6, a member of the A1 cluster, Roots515, a C1 cluster phage, and CBorch11, a member of the rare H1 cluster. Sibs6 and CBorch11 are siphoviridae phages and Roots515 is a podoviridae phage. The 50,210 base-pair genome of Sibs6 has a 63.8 % GC content and includes genes that suggest this phage is lysogenic, consistent with its plaque morphology and other A1 phages. Its left arm contains 38 forward genes and its right arm contains 58 reverse genes. We assigned functions to 41 of its 96 genes. The genome of Roots515 is 156,288 base pairs in size and has a 64.7% GC content. Its 271 genes are mostly forward and include 33 tRNA genes. We assigned functions to 50 of the protein-coding genes in Roots515. CBorch11’s genome is 68,508 base pairs in size and has a 57.6% GC content. CBorch11’s 93 genes are all forward genes and with presumed functions identified for 22 of them. Concurrent with annotation, structural proteins of 64/65 kDa from each phage were gel purified and used to immunize Balb/c mice. The polyclonal anti-phage protein antibodies raised against the 64/65 kDa proteins cross-react with proteins of similar size produced by the other two phages even though the phages are from different clusters. We are in the process of raising monoclonal antibodies to CBorch11 high titer lysate. Our initial work suggests that, while some of the anti-CBorch11 antibodies in the mice from which we are isolating monoclonal antibodies recognize proteins of similar sizes in Sibs6 and Roots515, other antibodies are specific for CBorch11 proteins alone. We hope to use the antibodies to understand the production of these proteins in the context of phage life cycles and to examine biochemical similarities and differences in phages from different clusters. In addition to this annotation and antibody work, we isolated, purified, and characterized 20 more mycobacteriophages that infect *M. smegmatis*. These came from seven different geographical locations. Plaque morphologies of these phages overlapped with previous isolates (2017) but, in general, were smaller. In contrast to 2017, none of the 2018 isolates appear to be potential lysogens.