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Hope College

Holland MI

Corresponding Faculty Member: Joseph Stukey (stukey@hope.edu)

Isolation and Genomic Analysis of New B3 and AI Mycobacteriophages

Dana Bacon, Alexa MacKersie, Diego Huby, Ella Pickett, Jayden Glazek, Zion Glen, Morgan Harkema, Stella Hill, Makenna Kayser, Madeline Kue, Meghan Marlette, Trevor Nelson, Liz Perez Silva, Madeline Webster, Joseph Stukey

At least 15 new mycobacteriophages were isolated from soil samples collected around the state of Michigan and parts of the United States. All were capable of infecting Mycobacterium smegmatis at 34°C and produced a variety of plaque morphologies based on size, shape, and clarity. Two mycobacteriophages, Chentzyk and Causa, were chosen for complete genome sequencing and comparative genomic analyses. Both produce small plaques with Causa’s taking at least 2 days to visibly form at 34°C. Further, and surprisingly, Causa genomic DNA did not digest with any tested restriction endonuclease, including HaeIII which targets the 4-bp sequence GGCC, a sequence expected to be plentiful in the Causa genome. Complete genome sequence of Chentzyk indicated it is a new member of Cluster B3. The Chentzyk genome is 69,383 bp long, has a GC content of 67.5%, contains 104 protein-coding genes, and no tRNA or tmRNA genes. Chentzyk\_36, an apparent HNH endonuclease, appears unique to the B3 mycobacteriophages. Similar copies of this gene are found in some other mycobacteriophage clusters, although not extensively, as well as in other actinobacteriophages that infect Rhodococcus, Gordonia, Corynebacterium, and Microbacterium. It has a GC content of 65.2%. The Causa genome sequence is similar to only one other mycobacteriophage – StAugustine. The pairing of Causa and StAugustine are the founding members of a new mycobacteriophage cluster – AI. The Causa genome is 101,485 bp long, has a GC content of 65.7%, contains about 180 protein-coding genes, approximately 17 tRNA genes, but no tmRNA genes. Comparative analysis with the annotated StAugustine genome revealed a possible explanation for the Causa restriction enzyme digest data – a suite of genes associated with the synthesis of 7-deazaguanosine base modifications. Although commonly found in tRNAs as queuosine, their inclusion in dsDNA has been shown to protect DNA from restriction endonuclease activity.