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Characterization and Genomic Analysis of Mycobacteriophage Wamburgrxpress, Including the Determination of the Essentiality of the Terminal 5 Kilobase Pairs for Plaque Formation

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Bacteriophages, viruses that specifically infect and kill bacterial cells, are the most numerous biological entities on Earth. Characterization of bacteriophages is necessary to understand their possible uses in medicine; for example, they could potentially be used as alternatives to antibiotics. Analysis of the genome of recently discovered bacteriophage, Wamburgrxpress, will provide new information about phage diversity by comparison to known phages. DNA sequencing revealed Wamburgrxpress contains a genome length of 74,392 bp, with 58.8% G/C content, belonging to phage cluster (L1) with a 10 bp 3’ overhang. Genome annotation was performed to identify 126 putative protein coding genes of which 36 had predicted functions. The genome additionally contains 9 tRNA genes. Transmission electron microscopy revealed a siphoviridae morphology, similar to other known L1 cluster phages. Determination of whether lysogen *Mycobacterium smegmatis* bacteria containing Wamburgrxpress prophages can be isolated by standard protocols further characterized our bacteriophage relative to other L1 cluster phages that are known to be temperate. Due to the presence of a putative integrase gene within the genome, it was predicted that Wamburgrxpress is also temperate. A key goal of our research was to identify whether the last 5,373 base pairs of its DNA are essential for productive *in vitro* infection of *M. smegmatis*. It was hypothesized that the terminal 5 kbp at the 3’ end of the genome are essential for infection (due to either the presence of essential genes or requirement of wildtype length to package its DNA correctly). Restriction digestions, agarose gel purification of the shortened phage genome, and electroporation into *M. smegmatis* of truncated DNA with and without a linker providing a cohesive end were performed. Plaques formed after plating with top agar were compared to those formed from electroporation of wildtype DNA.