Complete Genome Sequences of Microbacterium liquefaciens Phages Mercedes, Leafus, Nebulous, and Ixel

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ABSTRACT Microbacterium phages Mercedes, Leafus, Nebulous, and Ixel were isolated from soil in Rock Hill, SC. All are lytic phages with Siphoviridae morphotypes and similar genome sequence lengths that range from 40,200 bp to 42,000 bp. The four bacteriophages were isolated using the host Microbacterium liquefaciens.

An increase in the isolation and characterization of Microbacterium phages for potential therapeutic use, biotechnological applications, and host-pathogen evolutionary studies has recently occurred (1, 2). Here, Microbacterium liquefaciens LMG 16120 was used to isolate microbacteriophages in the soil at Winthrop University in Rock Hill, SC. This research is part of the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program (3). Protocols were provided by the HHMI SEA-PHAGES discovery guide (https://seaphagesphagediscoveryguide.helpdocsonline.com/home). Microbacterium phages Mercedes, Leafus, and Nebulous were isolated directly from sandy soil under grass and flowers, while Ixel was isolated from deeper moist black soil (see Table 1 for global positioning system [GPS] location coordinates) and required an initial enrichment step with M. liquefaciens. All phage went through two rounds of purification and were amplified in the bacterial host grown on peptone-yeast-calcium agar (PYCa) medium at 30°C. Transmission electron microscopy revealed that all four phages had Siphoviridae morphologies with long flexible tails (Fig. 1). Phage DNA was extracted from high-titer lysates using the Wizard DNA cleanup kit (Promega) and sequenced at the University of Pittsburgh. Libraries were constructed using the NEBNext Ultra II FS DNA library prep kit and sequenced using the Illumina MiSeq v3 sequencing platform; 150-bp single-end reads yielded 1,094-fold (Mercedes), 1,489-fold (Leafus), 707-fold (Nebulous), and 1,263-fold (Ixel) coverage of each genome (Table 1). The reads were assembled using Newbler v2.9 and checked for accuracy, coverage, and genomic termini using Consed v29 as previously described (4, 5). The results (genome size, GC content, and predicted number of genes and termini) and accession numbers (GenBank and SRA) are listed in Table 1. Using an online tool (https://phagesdb.org/genecontent/) at the PhagesDB database (6), all phages were assigned by gene content similarity (GCS) into cluster EA (35% or greater GCS). Within the cluster, Leafus was assigned into subcluster EA1, Nebulous into subcluster EAS, and Ixel into subcluster EA11 (1, 7).

For all bioinformatics analyses and software, default parameters were used. The genome sequences were annotated to identify open reading frames and predicted protein functions using DNA Master v5.22.3 (8), Glimmer v3.02 (9), GeneMark v2.5 (10), Staterator (8), Phamerator v3 (11), hhPred v2.07 (12), and BLASTp v2.7.1 (13). All four phages have the typical genomic architecture seen in the Microbacterium phage cluster EA genomes (1). Genes in the 5’ half of the genome sequence are forward encoded and include sequences for a portal protein, scaffolding protein, a major capsid protein, two tail assembly chaperones (predicted to be expressed using a programmed translational frameshift in Mercedes, Nebulous, and Ixel), and a tape measure protein.
majority of the 3’ half of the genome sequences are encoded on the reverse strand and include coding for DNA Pol I, MazG-like protein, and thymidylate synthase. None of the genome sequences contained genes for tRNAs, integrases, or immunity repressors, and so these phages are predicted to solely use the lytic pathway for replication.

Data availability. The individual GenBank and SRA accession numbers are listed in Table 1. The actinobacteriophage sequencing BioProject accession number is PRJNA488469.

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Phages Mercedes and Leafus were collected and initially annotated by undergraduate students in the 2019 SEA-PHAGES program at Winthrop University in Rock Hill, SC. Ixel and Nebulous were isolated by students Bethany Wise and Allyssa Lewis, respectively, outside the course. Allyssa Lewis contributed to the annotation of Nebulous. We also thank Julian Smith, the director of microscopy at Winthrop, for help in obtaining transmission electron microscopy (TEM) micrographs.

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REFERENCES


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<th>Phage name</th>
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<th>SRA accession no.</th>
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<th>No. of reads (thousands)</th>
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<th>Genome ends</th>
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</table>

FIG 1 Transmission electron micrographs of *Microbacterium* phages Ixel (A), Leafus (B), Nebulous (C), and Mercedes (D). Phage lysates were negatively stained with 1% uranyl acetate.


