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GENOME SEQUENCES

Complete Genome Sequence of Bacteriophage Loca, Isolated on a *Microbacterium foliorum* Culture

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ABSTRACT Microbacteriophage Loca was extracted from a shopping cart handle swab sample in Stephenville, TX, and isolated on a *Microbacterium foliorum* NRRL-24224 culture. The 17,475-bp double-stranded DNA genome contains 25 predicted protein-coding genes and has >96% nucleotide identity to bacteriophages Quaker and Livingwater.

Microbacterium bacteriophages are genetically diverse and composed of several types of genomic architectures containing multiple genes of unknown function (1). To expand our knowledge of the diversity of microbacteriophages isolated from central and north Texas (2–4), we report the genome sequence of microbacteriophage Loca, collected from a swab sample of a shopping cart handle in Stephenville, TX (global positioning system [GPS] coordinates, 32.206238 N, 98.23701 W). The sample was submerged in peptone-yeast extract-calcium (PYCa) liquid medium and incubated while shaking for 2 h at 220 rpm and 29°C. The supernatant was filtered through a 0.22-μm filter and incubated with the bacterial host strain *Microbacterium foliorum* NRRL B-24224 in PYCa liquid medium for 6 days at 29°C (5, 6). The bacteria were pelleted and the supernatant passed through 0.22-μm filters. The filtrates were 10-fold serially diluted in phage buffer (10 mM Tris [pH 7.5], 10 mM $MgSO_4$, 68 mM NaCl, 1 mM $CaCl_2$, 10% glycerol) and incubated with *M. foliorum* in a soft agar overlay on PYCa agar plates for 48 h at 29°C. Bacteriophage replication formed clear, circular plaques approximately 5 mm in diameter within the soft agar overlay. Loca was isolated by two rounds of picking a single, well-separated plaque, followed by 10-fold serial dilution of the bacteriophage sample and plating it with *M. foliorum* as before. High-titer lysates were prepared by flooding "webbed" plates with phage buffer overnight at 4°C, as described in *Phage Discovery Guide* (5). Negative-staining transmission electron microscopy showed that Loca exhibited *Siphoviridae* morphology (Fig. 1), and ImageJ v1.53m (7) was used to measure an approximate tail length of 105 nm and capsid diameter of 40 nm ($n = 9$).

Genomic DNA was extracted from the high-titer lysate using a modified zinc chloride precipitation method (5, 8). The Pittsburgh Bacteriophage Institute prepared sequencing libraries using the NEBNext Ultra II DNA library prep kit (New England Biolabs, Ipswich, MA) and sequenced them using an Illumina MiSeq instrument to produce 20,276 single-end 150-bp reads. The raw reads were assembled using Newbler v2.9 to generate a single contig with 75× coverage that was checked for completeness and genomic termini using Consed v29 (9, 10). The 17,475-bp double-stranded DNA genome contains 9-nucleotide 3′ single-stranded cohesive ends (5′-CCCGCCCCA-3′) and 68.7% G+C content.

A BLASTn (11) query of the sequence against the nonredundant/nucleotide (nr/nt) database returned >96% nucleotide sequence identity to the cluster EE bacteriophages Quaker (GenBank accession number MH371111) and Livingwater (MT498040) (1). Auto-annotation with GLIMMER v3.02 (12) and GeneMark v2.5p (13) was manually refined using Phamerator (14), PECAAN, and DNA Master v5.23.3 (http://phagesdb.org/DNAMaster/). No tRNA genes were identified using Aragorn v1.1 (15) or tRNAscan-SE v2.0 (16). Putative functions for 18 of

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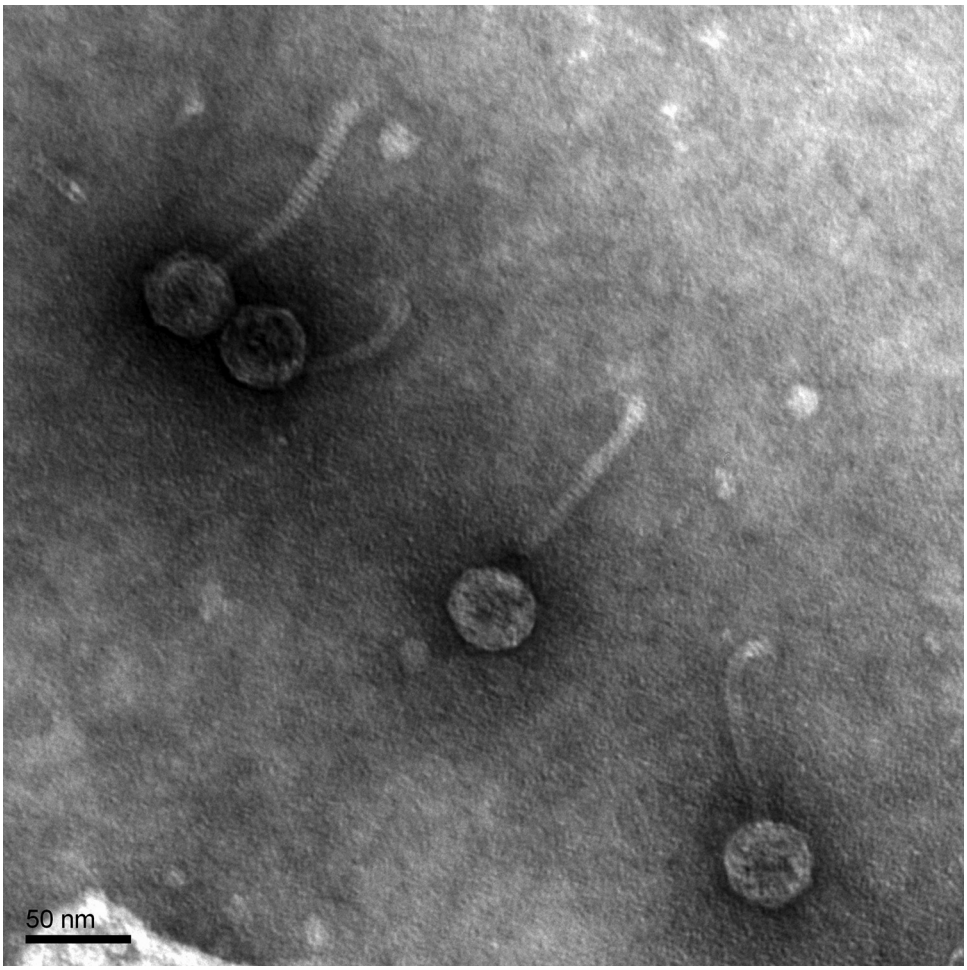

FIG 1 Transmission electron microscopy of microbacteriophage Loca. High-titer lysate was placed on a 300-mesh copper grid and negatively stained with uranyl acetate. Imaging with an FEI Tecnai G2 Spirit BioTWIN transmission electron microscope (NL1.160G) showed an approximate capsid diameter of 40 nm, a tail length of 105 nm (n = 9), and *Siphoviridae* morphology.

25 predicted protein-coding genes were assigned using BLASTp (11) and HHpred (17). All tools were run with default parameters. Rightward-transcribed genes 1 to 19 encode virion structural and assembly proteins and terminase and endolysin proteins. Leftward-transcribed genes 20 to 22 encode an Lrs2-like DNA-bridging protein and two helix-turn-helix DNA binding domains. Rightward transcribed genes 23 to 25 encode a helix-turn-helix DNA binding domain and HNH endonuclease.

Data availability. The sequence has been deposited under GenBank accession number ON260814 and the raw reads under SRA accession number SRX14483214.

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