Complete Genome Sequence of Mycobacteriophage Joy99

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ABSTRACT   Joy99 is a siphoviral mycobacteriophage with a 59,837-base pair double-stranded DNA genome and is predicted to contain 97 protein-coding genes and a single tRNA gene. Joy99 was isolated in Saint Louis, MO, and annotated by students at Bluff Dale High School in community engagement with Tarleton State University.

In 2019, 10 million *Mycobacterium tuberculosis* infections occurred worldwide (1). An estimated half million infections were rifampin-resistant tuberculosis, and 206,030 were multidrug-resistant tuberculosis (MDR-TB), a 10% increase from 2018 (1). Characterized bacteriophages could offer an alternative treatment option for MDR-TB (2, 3). Mycobacteriophage Joy99 was discovered in soil from a municipal mulch pile in Saint Louis, MO (global positioning system [GPS] coordinates 38.664567 N, 90.3179 W) (4, 5). Soil samples were incubated in 7H9 liquid medium at 37°C for 2 h before the supernatant was centrifuged, filtered through a 0.22-μm filter, and incubated with *Mycobacterium smegmatis* mc² 155 at 37°C on Luria agar plates. Joy99 was isolated by two rounds of picking a single, well-separated plaque, followed by diluting the bacteriophage sample in a 10-fold dilution series and plating it with *M. smegmatis* mc² 155 (6).

Joy99 plaques formed a three-ring morphology with a clear center spot, thin middle ring, and turbid outer ring. Negative-staining transmission electron microscopy with 1% uranyl acetate on a carbon type B 300-mesh grid showed *Siphoviridae* morphology (4). ImageJ v1.53h (7) measured a 75-nm-wide isometric capsid and 180-nm-long tail (Fig. 1). High-titer lysate was obtained by flooding "webbed" plates, as described in the Phage Discovery Guide, and DNA extraction was performed at Washington University using the Promega Wizard DNA clean-up system (8). Sequencing libraries were prepared from genomic DNA using a 454 DNA
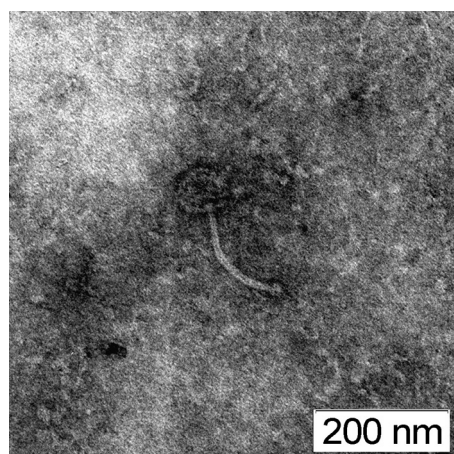
FIG 1 Transmission electron microscopy (TEM) of mycobacteriophage Joy99. High-titer lysate on a carbon type B 300-mesh grid was negatively stained with 1% uranyl acetate. A TEM micrograph of mycobacteriophage Joy99 shows an approximately 75-nm-diameter nonenveloped icosahedral capsid and a 180-nm flexible noncontractile tail.

TABLE 1 Characteristics of similar bacteriophages with *M. smegmatis* as a host[a]

Name	GenBank accession no.	Genome size (bp)	GC content (%)	No. of ORFs[b]	No. of tRNAs	Identity to Joy99 (%)
Joy99	MH536822	59,837	66.6	97	1	
BellaDonna	MH697578	59,708	66.6	97	1	99.46
Dalmuri	MH727544	59,708	66.5	97	1	99.45
Inky	MN369746	59,708	66.5	96	1	99.44
Jekyll	MF140412	59,708	66.6	97	1	99.44
CREW	KY380102	59,707	66.6	95	1	99.44

[a] Similarity defined as an identity of >95%.
[b] ORFs, open reading frames.

library prep kit and sequenced with a Roche 454 GS FLX instrument at the Pittsburgh Bacteriophage Institute to approximately 157-fold coverage from 17,587 total single-end reads with an average read length of 165 bases (9). The single-bacteriophage contig assembly was performed using Newbler v2.7, and the assembly was checked for completeness, accuracy, and genome termini using Consed v22.0 software (9, 10). Joy99 has a linear double-stranded DNA genome that is 59,837 bp long with 66.6% G+C content and an 11-base 3′ sticky overhang composed of 5′-CTCGTAGGCAT-3′.

Whole-genome nucleotide alignment using BLASTn (https://blast.ncbi.nlm.nih.gov/) indicated that Joy99 has nucleotide sequence identity of more than 99% with other subcluster K1 bacteriophages (Table 1) (11). Initial auto-annotations were generated using Glimmer v3.02 (12) and GeneMark v2.5p (13) and were revised manually using DNA Master v5.23.2 (http://phagesdb.org/DNAMaster/) and PECAAN (https://pecaan.kbrinsgd.org/). Gene functions were assigned using HHpred v3.0beta (14, 15) and BLASTp (11). The tRNA was characterized using ARAGORN v1.2.38 (16) and tRNAscan-SE v2.0 (17). All tools were run with default parameters. The Joy99 genome contains 97 predicted protein-coding genes and a single tRNA gene for tryptophan; 14.3% of K1 bacteriophages encode a tRNA. The start codon usage is 58.7% AUG, 40.2% GUG, and 0.1% UUG. Like other K1 bacteriophage genomes, Joy99 contains genes for virion assembly and structure, lysis proteins, host integration and excision proteins, DNA primase, RusA-like resolvase, and RtcB-like ligase.

Data availability. The Joy99 genome sequence is available under GenBank accession number MH536822. The raw reads are available under SRA accession number SRX4721439.

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