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GENOME SEQUENCES

Complete Genome Sequences of Mycobacteriophages OKaNui and DroogsArmy

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ABSTRACT Mycobacteriophages OKaNui and DroogsArmy were isolated from soil using the bacterial host *Mycobacterium smegmatis* mc²155, which belongs to the phylum *Actinobacteria*. OKaNui was discovered in east Mississippi and DroogsArmy in west Alabama in the United States. The genomes of OKaNui and DroogsArmy were 51,424 bp and 53,254 bp long, respectively.

Among biological agents, bacteriophages (phages) are the most populous and ubiquitous in the environment (1). The phages named OKaNui and DroogsArmy were isolated from the bacterial host *Mycobacterium smegmatis* mc²155. Most *Mycobacteria* species are saprophytic and reside in the soil or water or on plants (2). Although *M. smegmatis* is a nonpathogenic bacterium, an understanding of phage infection in this strain may contribute to advances in phage therapy for other *Mycobacteria* species. *M. smegmatis* may be used as a delivery system for phages intended for infection of *M. avium* and *M. tuberculosis* (3). Phages isolated from *M. smegmatis* have be used in the experimental treatment of closely related hosts, including *M. ulcerans* (4). OKaNui was discovered in Meridian, Mississippi, in moist soil in a shaded area, and DroogsArmy was found in Lisman, Alabama, in a dark-colored, moist soil (Table 1).

The soil samples were inoculated with the host bacterium and incubated at 37°C with shaking in 7H9 liquid medium (5). A filtrate was then plated on a lawn of *M. smegmatis* mc²155 along with a negative control (5). The formation of plaques following incubation at 37°C were indicative of phage presence. Isolates were purified using serial dilutions and a filtered high-titer lysate (HTL) collected from webbed plates (5). Phage genomic DNA was extracted using the Wizard DNA cleanup system with modified protocols (Promega, Madison, WI) (5). DNA libraries were built and pooled for sequencing using the NEBNext Ultra II FS kit with dual-indexed barcoding (New England BioLabs, Ipswich, MA). Sequencing was performed using the Illumina MiSeq platform at the Pittsburgh Bacteriophage Institute. The genome lengths, G+C content, and coverage depths are listed in Table 1. OKaNui yielded ~316,000 single-end 150-base reads, and DroogsArmy yielded ~410,000 reads. Both genomes displayed defined ends with 10-bp overhangs (CGGCCGGTAA). Assembly was performed using Newbler 2.9 with default settings (6). A single contig for each genome was produced and used to determine the genome ends; the beginning of each genome was chosen based on similar genomes. These were then checked for completeness and accuracy using Consed 2.0 (7).

Genome annotation was performed using the Phage Evidence Collection and Annotation Network (PECAAN; https://discover.kbrinsgd.org/) and DNA Master 5.23.3 (cobamide2.bio.pitt.edu/computer.htm). We used the following programs to determine gene presence, functions, and start sites: NCBI BLAST, Phamerator (https://phamerator.org/), PhagesDB BLAST, HHPred 3.0, Starterator (https://github.com/SEA-PHAGES/

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TABLE 1 Characteristics and accession numbers of genomes

Bacteriophage isolate	Accession no.	Genome size (bp)	No. of protein CDSs[a]	No. of tRNAs	G+C content (%)	Location		Read coverage (×)	Phage life cycle	Closest relative	
						County, state	Coordinates			Isolate (GenBank accession no.)	Nucleotide identity (coverages [%])
OKaNui	MT490373.1	51,424	87	0	63.9	Lauderdale County, MS	32.59, −88.19	874	Lysogenic	Kingmustik0402 (MK359301.1)	99.2, 99.0
DroogsArmy	MT553337.1	53,254	83	1	63.0	Choctaw County, AL	32.28, −88.28	1,097	Lysogenic	Timshel (NC_041983.1)	98.9, 95.0

[a] CDSs, coding DNA sequences.

starterator), and GeneMark 3.25 (8–11). Detection and trimming of tRNAs were performed in ARAGORN 1.2.38 and tRNAscan-SE 2.0 (12, 13). The complete numbers of protein coding genes and tRNAs are reported in Table 1.

When plated with the host, OKaNui produced relatively large plaques with a halo, and DroogsArmy produced small plaques. Both OKaNui and DroogsArmy were identified as lysogenic phages because they produced turbid plaques, and the genomes contained genes for lysogeny (14). Transmission electron microscopy and genome analysis indicated that the phages share the *Siphoviridae* morphotype (5). Based on nucleotide similarity and synteny across the genome, OKaNui has been placed in the A4 subcluster of mycobacteriophages and DroogsArmy in A7 (15).

Data availability. The complete genome sequences of OKaNui and DroogsArmy are available from GenBank under the accession numbers MT490373.1 and MT553337.1, respectively. The raw Illumina reads for OKaNui and DroogsArmy are available on NCBI's Sequence Read Archive under accession numbers SRX8622883 and SRX8622882, respectively.

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