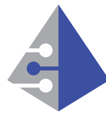
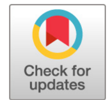


GENOME SEQUENCES



Complete Genome Sequence of *Serratia* Phage Muldoon

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ABSTRACT *Serratia marcescens* is a ubiquitous Gram-negative bacterium that is linked with emerging opportunistic infections. In this report, we describe the isolation and annotation of an *S. marcescens* myophage called Muldoon. Related to T4-like phages, such as *Serratia* phage PS2, Muldoon contains 257 predicted protein-coding genes and 4 tRNA genes.

Serratia marcescens is an often pigmented Gram-negative member of the *Enterobacteriaceae* family (1). An increasing incidence of human disease caused by this pathogen is linked with multidrug resistance (2). Here, we describe the isolation, genome sequencing, and annotation of bacteriophage Muldoon, which targets *S. marcescens*.

Phage Muldoon was isolated from filtered (0.2-μm pore size) wastewater treatment plant samples collected in College Station, TX, by growth on *Serratia marcescens* D1 (catalog no. 8887172; Ward's Science). The host was cultivated aerobically in LB (BD) at 30°C and 37°C. Muldoon was propagated by the soft-agar overlay method (3). Illumina TruSeq libraries were generated with a Nano low-throughput kit after DNA was purified, as described in the shotgun library preparation protocol by Summer (4), and phage Muldoon was sequenced on an Illumina MiSeq platform with paired-end 250-bp reads using v2 500-cycle chemistry. FastQC was used to control the quality of 565,076 total sequence reads (www.bioinformatics.babraham.ac.uk/projects/fastqc). The FastX Toolkit v0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/) was used for trimming before assembly with default parameters using SPAdes v.3.5.0 (5). The result was a contig with 69.2× coverage. To ensure that the complete termini were present, PCR products (forward, 5′-GTCACGATTTCCCTGCTATCT-3′; reverse, 5′-GCCGAATTTGCGTACGTTTAC-3′) amplified off the contig ends were Sanger sequenced. Structural annotation was carried out with GLIMMER v3.0 and MetaGeneAnnotator v1.0 for protein-coding genes and with ARAGORN v2.36 for tRNA genes (6–8). Rho-independent termination sites were annotated using TransTermHP v2.09 (9). Functional annotations were guided by results from InterProScan v5.33-72, BLAST v2.2.31, and TMHMM v2.0 analyses (10–12). BLAST searches were conducted with the NCBI nonredundant, UniProtKB Swiss-Prot, and UniProtKB TrEMBL databases at a maximum expectation value of 0.001 (13). Whole-genome comparisons were performed by the progressiveMauve v2.4.0 algorithm (14). Genomic terminus type was predicted with PhageTerm (15). All of the annotation tools listed above are in the Galaxy and Web Apollo instances hosted by the Center for Phage Technology at https://cpt.tamu.edu/galaxy-pub/ (16, 17). To determine morphology, Muldoon samples were negatively stained with 2% (wt/vol) uranyl acetate and viewed by transmission electron microscopy at the Texas A&M Microscopy and Imaging Center (18).

Myophage Muldoon has a 167,457-bp genome with a G+C content of 42%. With 257 predicted protein-coding genes and 4 tRNA genes, Muldoon has a 93% coding density. The genome was predicted to have permuted termini, indicating that this phage uses a T4-like packaging mechanism, and it was therefore reopened at the junction between its equivalents of the *rIIA* and *rIIB* genes to be syntenic with phage

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T4 (GenBank accession no. NC_000866). Unlike the T4 genome, Muldoon has no detectable introns. Phage Muldoon has its highest identity with *Serratia* phage PS2 (GenBank accession no. KJ025957), with 77.91% nucleotide identity and 253 similar proteins. Phage PS2 has a similarly large genome of 167,276 bp and has an identical number of tRNAs (19).

Data availability. The genome sequence and associated data for phage Muldoon were deposited under GenBank accession no. MN095771, BioProject accession no. PRJNA222858, SRA accession no. SRR8893603, and BioSample accession no. SAMN11414488.

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