

# Complete Genome Sequence of *Citrobacter freundii* Myophage Miller

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***Citrobacter freundii* is a Gram-negative, opportunistic pathogen that can be fatal to newborns or immunocompromised patients. Bacteriophages against this bacterium can be useful for therapeutic purposes. Here, we describe the complete genome and the key features of the pseudo T-even *C. freundii* bacteriophage Miller.**

Received 1 December 2014 Accepted 5 December 2014 Published 15 January 2015

**Citation** Hwang K, Luna AJ, Hernandez AC, Kutty Everett GF. 2015. Complete genome sequence of *Citrobacter freundii* myophage Miller. *Genome Announc.* 3(1):e01425-14. doi:10.1128/genomeA.01425-14.

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*Citrobacter freundii* is a Gram-negative ubiquitous bacterium. It is a member of the family Enterobacteriaceae and is associated with fatal neonatal meningitis and various nosocomial infections (1). The treatment of such conditions is difficult due to the broad range of antibiotic resistance expressed by many strains of the bacterium (2). Moreover, it has been proposed that the *C. freundii* may play a role in disseminating antibiotic resistance genes to other microbial communities in soil (3). Bacteriophages against *C. freundii* could be used to quell the growing problem of this pathogenic bacterium.

Bacteriophage Miller was isolated from a sewage sample collected in Bryan, TX, USA. Phage DNA was sequenced in an Illumina MiSeq 250-bp paired-end run with a 550-bp insert library at the Genomic Sequencing and Analysis Facility at the University of Texas (Austin, TX, USA). Quality controlled, trimmed reads were assembled to a single contig at 30.1 fold coverage using Velvet version 1.2.10. Contigs were confirmed to be complete by PCR. Genes were predicted using GeneMarkS (4) and corrected using software tools available on the Center for Phage Technology (CPT) Galaxy instance (<https://cpt.tamu.edu/galaxy-public/>). Morphology was determined using transmission electron microscopy performed at the Texas A&M University Microscopy and Imaging Center.

Miller is an RB43-like, morphotype A2 myophage that shares 88.2% homology with RB43 (NC\_007023) as determined by Emboss Stretcher (5, 6). The genomic differences are largely attributed to hypothetical conserved/novel genes of unknown function. Unlike RB43, which has a fused *rIIa* and *rIIb* gene, Miller has separate *rIIa* and *rIIb* genes that share an overlapping start/stop codon. As with RB43 and other pseudo T-even phages, the genome of Miller is opened to *rIIb* (7). Miller has a 178,171-base pair genome, a coding density of 95.6%, 43.13% G+C content, and 277 predicted genes. The G+C content is comparable to RB43 (43.2%). Of the 277 predicted coding sequences, 170 are hypothetical conserved/novel, and 99 were given a putative function based on BLASTp and InterPro analysis (8, 9). Miller encodes one homing endonuclease with an AP2 DNA-binding domain compared to the three AP2 domain containing HNH homing endonucleases of RB43. Like RB43, Miller encodes a single tRNA gene. In RB43, the tRNA is an Ile where as in Miller, it is a Met as has been reported in RB43-like *Escherichia coli* phage Lw1 (7).

Overall, the genome of Miller is syntenic with that of RB43. Pseudo T-even-like genes encoding the proteins for biosynthesis, DNA replication, recombination, morphogenesis, DNA packaging, and lysis were annotated. A T-even-like lysis cassette was identified including a class III holin, inner and outer membrane spanins, an *rI*-like antiholin, and an endolysin. Unlike T4, which encodes a muramidase (EC 3.2.1.17); the endolysin of Miller is a peptidase (EC 3.4.16.4) as with other RB43-like phages. An additional peptidoglycan-binding protein was identified by its LysM domain (IPR018392), but the role of this protein in the phage infection is unknown.

**Nucleotide sequence accession number.** The genome sequence of phage Miller was contributed as accession no. [KM236237](https://www.ncbi.nlm.nih.gov/nuclot/KM236237) to GenBank.

## ACKNOWLEDGMENTS

This work was supported primarily by funding from award number EF-0949351, “Whole Phage Genomics: A Student-Based Approach,” from the National Science Foundation. Additional support came from the Center for Phage Technology, an Initial University Multidisciplinary Research Initiative supported by Texas A&M University and Texas AgriLife, and from the Department of Biochemistry and Biophysics.

The authors are grateful for the advice and support of the CPT staff.

This Announcement was prepared in partial fulfillment of the requirements for BICH464 Phage Genomics, an undergraduate course at Texas A&M University.

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