genomeA
Journals.ASM.org

Complete Genome Sequence of *Citrobacter freundii* Myophage Mordin

Jingwen Guan, Jeffrey D. Snowden, Jesse L. Cahill, Eric S. Rasche, Gabriel F. Kuty Everett

Center for Phage Technology, Texas A&M University, College Station, Texas, USA

***Citrobacter freundii* is a Gram-negative opportunistic pathogen that is associated with urinary tract infections. Bacteriophages infecting *C. freundii* can be used as an effective treatment to fight these infections. Here, we announce the complete genome sequence of the *C. freundii* Felix O1-like myophage Mordin and describe its features.**

Received 1 September 2015 **Accepted** 9 September 2015 **Published** 15 October 2015

Citation Guan J, Snowden JD, Cahill JL, Rasche ES, Kuty Everett GF. 2015. Complete genome sequence of *Citrobacter freundii* myophage Mordin. Genome Announc 3(5): e01203-15. doi:10.1128/genomeA.01203-15.

Address correspondence to Gabriel F. Kuty Everett, cpt@tamu.edu.

Citrobacter freundii is a member of the family *Enterobacteriaceae*. *C. freundii* is a significant cause of opportunistic infections, such as neonatal meningitis, septicemia, and brain abscesses (1). Due to increasing antibiotic resistance among *C. freundii* strains (2), bacteriophages may be an alternative therapy against this pathogen. Here, we present the complete genome sequence of *C. freundii* Felix O1-like myophage Mordin.

Bacteriophage Mordin was isolated from a water sample collected at College Station, TX. 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). Quality-controlled trimmed reads were assembled to a single contig of circular assembly at 65-fold coverage using SPAdes version 3.5.0 (3). The contig was confirmed to be complete by PCR using primers that face the upstream and downstream ends of the contig. The products from PCR amplification of the junctions of concatemeric molecules were sequenced by Sanger sequencing (Eton Bioscience, San Diego, CA). 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-pub/). Morphology was determined using transmission electron microscopy performed at the Texas A&M University Microscopy and Imaging Center.

Mordin has an 89,596-bp double-stranded DNA (dsDNA) genome containing 138 predicted coding sequences. It has a coding density of 89.3% and a G+C content of 38.8%. The G+C content of Mordin is similar to that of *Salmonella* phage Felix O1 (accession no. NC_005282) (39.0%) (5), but it is significantly lower than that of *C. freundii* (51.61%) (6). The *Salmonella* G+C content (approximately 52%) is similar to that of *Citrobacter* (7). A low G+C content compared to its host seems to be a common feature of Felix O1-like phages (5, 8). Of the 138 predicted coding sequences, 106 are hypothetical novel or conserved genes, and 32 were given a putative function based on BLASTp and InterProScan analyses (9, 10). Sequence analysis using Emboss Stretcher showed that Mordin shares 46.7% and 48.8% nucleotide sequence identity across the genome with Felix O1 and *Escherichia* phage wV8 (accession no. NC_012749), respectively (11). Mordin is syntenic with Felix O1, and despite low sequence identity, 114 of 138 (82.6%) putative coding sequences are similar, according to CoreGenes (12). Most of the differences between the two phages occur in hypothetical proteins of unknown function. Mordin contains 25 tRNA genes, similar to the 22 tRNAs identified in Felix O1 (5). As with Felix O1, Mordin contains *rIIA* and *rIIB* genes and was opened just upstream of the *rIIA* homolog for annotation purposes. Mordin encodes a single HNH homing endonuclease, whereas five have been identified in Felix O1. Interestingly, a 26-bp repeat (consensus, 5′-CCAACAATCCTAAACTGGAGAA TCTA-3′), reminiscent of start-associated sequences (SASs) described in cluster K mycobacteriophages, was identified upstream of the translational start of 8 hypothetical conserved/novel genes on the left arm of the genome (13). The role of SASs in gene expression is currently unknown (14).

Nucleotide sequence accession number. The genome sequence of phage Mordin was deposited in GenBank under the accession no. KT363872.

ACKNOWLEDGMENTS

This work was supported primarily by funding from award no. 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 at Texas A&M University.

We thank the CPT staff for their advice and support. This announcement was prepared in partial fulfillment of the requirements for BICH464 Phage Genomics, an undergraduate course at Texas A&M University.

REFERENCES

1. **Badger JL, Stins MF, Kim KS.** 1999. *Citrobacter freundii* invades and replicates in human brain microvascular endothelial cells. Infect Immun **67:**4208–4215.
2. **Lockhart SR, Abramson MA, Beekmann SE, Gallagher G, Riedel S, Diekema DJ, Quinn JP, Doern GV.** 2007. Antimicrobial resistance among gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. J Clin Microbiol **45:** 3352–3359. http://dx.doi.org/10.1128/JCM.01284-07.
3. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA.** 2012. SPAdes: a new genome assembly algorithm and its applications to single-

cell sequencing. J Comput Biol **19:**455–477. http://dx.doi.org/10.1089/cmb.2012.0021.

4. **Besemer J, Lomsadze A, Borodovsky M.** 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res **29:**2607–2618. http://dx.doi.org/10.1093/nar/29.12.2607.
5. **Whichard JM, Weigt LA, Borris DJ, Li LL, Zhang Q, Kapur V, Pierson FW, Lingohr EJ, She YM, Kropinski AM, Sriranganathan N.** 2010. Complete genomic sequence of bacteriophage Felix O1. Viruses **2:**710–730. http://dx.doi.org/10.3390/v2030710.
6. **Kumar S, Kaur C, Kimura K, Takeo M, Raghava GP, Mayilraj S.** 2013. Draft genome sequence of the type species of the genus *Citrobacter*, *Citrobacter freundii* MTCC 1658. Genome Announc **1**(1):e00120-12. http://dx.doi.org/10.1128/genomeA.00120-12.
7. **Papanikolaou N, Trachana K, Theodosiou T, Promponas VJ, Iliopoulos I.** 2009. Gene socialization: gene order, GC content and gene silencing in *Salmonella*. BMC Genomics **10:**597. http://dx.doi.org/10.1186/1471-2164-10-597.
8. **Villegas A, She YM, Kropinski AM, Lingohr EJ, Mazzocco A, Ojha S, Waddell TE, Ackermann HW, Moyles DM, Ahmed R, Johnson RP.** 2009. The genome and proteome of a virulent *Escherichia coli* O157:H7 bacteriophage closely resembling *Salmonella* phage Felix O1. Virol J **6:**41. http://dx.doi.org/10.1186/1743-422X-6-41.
9. **Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL.** 2009. BLAST+: architecture and applications. BMC Bioinformatics **10:**421. http://dx.doi.org/10.1186/1471-2105-10-421.
10. **Hunter S, Apweiler R, Attwood TK, Bairoch A, Bateman A, Binns D, Bork P, Das U, Daugherty L, Duquenne L, Finn RD, Gough J, Haft D, Hulo N, Kahn D, Kelly E, Laugraud A, Letunic I, Lonsdale D, Lopez R, Madera M, Maslen J, McAnulla C, McDowall J, Mistry J, Mitchell A, Mulder N, Natale D, Orengo C, Quinn AF, Selengut JD, Sigrist CJ, Thimma M, Thomas PD, Valentin F, Wilson D, Wu CH, Yeats C.** 2009. InterPro: the integrative protein signature database. Nucleic Acids Res **37:**D211–D215. http://dx.doi.org/10.1093/nar/gkn785.
11. **Myers EW, Miller W.** 1988. Optimal alignments in linear space. Comput Appl Biosci **4:**11–17. http://dx.doi.org/10.1093/bioinformatics/4.1.11.
12. **Zafar N, Mazumder R, Seto D.** 2002. CoreGenes: a computational tool for identifying and cataloging "core" genes in a set of small genomes. BMC Bioinformatics **3:**12. http://dx.doi.org/10.1186/1471-2105-3-12.
13. **Pope WH, Ferreira CM, Jacobs-Sera D, Benjamin RC, Davis AJ, DeJong RJ, Elgin SC, Guilfoile FR, Forsyth MH, Harris AD, Harvey SE, Hughes LE, Hynes PM, Jackson AS, Jalal MD, MacMurray EA, Manley CM, McDonough MJ, Mosier JL, Osterbann LJ, Rabinowitz HS, Rhyan CN, Russell DA, Saha MS, Shaffer CD, Simon SE, Sims EF, Tovar IG, Weisser EG, Wertz JT, Weston-Hafer KA, Williamson KE, Zhang B, Cresawn SG, Jain P, Piuri M, Jacobs WR, Jr, Hendrix RW, Hatfull GF.** 2011. Cluster K mycobacteriophages: insights into the evolutionary origins of mycobacteriophage TM4. PLoS One **6:**e26750. http://dx.doi.org/10.1371/journal.pone.0026750.
14. **Pope WH, Carter JT, Pope WH, Carter JT, Dasher KL, Haynberg MC, Reddi A, Shedlock KA, Lapin JS, Prout AK, Grubb SR, Warner MH, Bowman CA, Russell DA, Hatfull GF.** 2015. Genome sequence of a newly isolated mycobacteriophage, ShedlockHolmes. Genome Announc **3**(3):e00597-13. http://dx.doi.org/10.1128/genomeA.00597-15.