Complete Genome Sequence of *Bacillus megaterium* Myophage Moonbeam

Joshua N. Cadungog, Brontee E. Khatemi, Adriana C. Hernandez, Gabriel F. Kuty Everett

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

Moonbeam is a newly isolated myophage of *Bacillus megaterium*, a common Gram-positive bacterium that is routinely used for large-scale protein production. Bacteriophages have potential to be useful tools for industrial applications. Here, we describe the complete genome of Moonbeam and describe its features.

**Bacillus megaterium** is a soil-dwelling bacterium that is commonly used for the production of recombinant proteins (1). Additionally, it is being investigated for use in biomineralization and bioremediation of soil contaminated with petrochemicals (2, 3). *B. megaterium* phages could provide a useful tool for manipulating these systems. Here, we describe a novel myophage, Moonbeam, which was isolated against the asporogenic *B. megaterium* strain Km sp.

Bacteriophage Moonbeam was isolated from a soil sample collected in 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, USA). Quality controlled, trimmed reads were assembled to a single contig at 32.6-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.

Bacteriophage Moonbeam has a 161,239-bp double-stranded DNA genome, including 1,980-bp terminal repeats, as determined by processing of raw sequencing data using PAUSE (https://cpt.tamu.edu/pause/). The unit genome has a GC content of 40.2%, a coding density of 90.6%, and 3 tRNA genes. Of the 231 predicted genes, 79 are hypothetical novel, and 88 are hypothetical conserved. Comparison using EMBOSS Stretcher revealed 47.0% identity with paradigm phage SPO1 (NC_011421) (5).

Several functional proteins were identified using BLASTp and InterProScan analyses (6, 7). Genes encoding structural proteins include a capsid protein, portal, prohead protease, tail proteins, tail chaperones, tape measure protein, tail proteins, and multiple components of the baseplate. The tail chaperone had an unusual +1 frameshift to its secondary product, where most Caudovirales use a −1 frameshift to encode their secondary tail chaperone (8). Notably, the terminase is interrupted by two group I introns containing embedded homing endonuclease genes. Homing endonucleases are commonly embedded within group I introns, which catalyze their own splicing from an mRNA transcript (9, 10). An interruption of a terminase by a group I intron has also been reported in *Lactobacillus* phage LL-H (11). The terminase is homologous to the terminases of several *Bacillus* phages, including Bcp1, 1102phi1-3, and Spock (12–14). Genes encoding replication fork components, including primase, helicase, and DNA polymerase, were identified, as were several DNA-binding proteins. As in SPO1, the DNA polymerase gene of Moonbeam is interrupted by group I intron-embedded homing endonucleases, although SPO1 DNA polymerase contains one intron while Moonbeam encodes two (15). Genes for biosynthesis proteins were present, such as thymidylate synthase, dihydrofolate reductase, ribonucleotide reductase, and dUTPase. A class-II holin gene (two transmembrane domains in an N-in, C-in topology) was identified, although the antiholin was not found (16). Moonbeam encodes an FtsK/SpoIIIIE DNA pump, although its role in the phage infection is unknown (17).

**Nucleotide sequence accession number.** The genome sequence of phage Moonbeam was contributed to GenBank with the accession number KM236246.

**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.

We 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.

**REFERENCES**

1. Biedendieck R, Borgmeier C, Bunk B, Stammen S, Scherling C, Meinhardt F, Wittmann C, Jahn D. 2011. Systems biology of recombinant protein production using *Bacillus megaterium*. Methods Enzymol 500:165–195. http://dx.doi.org/10.1016/B978-0-12-385118-5.00010-4.
2. Cerqueira VS, Hollenbach EB, Maboni F, Camargo FA, Peralba Mdo C, Bento FM. 2012. Bioprospection and selection of bacteria isolated from environments contaminated with petrochemical residues for application in bioremediation. World J Microbiol Biotechnol 28:1203–1222. http://dx.doi.org/10.1007/s11274-011-0923-z.

3. Dhami NK, Reddy MS, Mukherjee A. 2013. Bacillus megaterium mediated mineralization of calcium carbonate as biogenic surface treatment of green building materials. World J Microbiol Biotechnol 29:2397–2406. http://dx.doi.org/10.1007/s11274-013-1408-z.

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. Myers EW, Miller W. 1988. Optimal alignments in linear space. Comput Appl Biosci 4:11–17.

6. 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 K, McAnulla C, McDowall J, Mistry J, Mitchell A, Mulder N, Natale D, Orenço C, Quinn AF, Selengut JD, Sigrist CJ, Slima M, Thomas PD, Valentin F, Wilson D, Yeats C. 2009. Interpro: the integrative protein signature database. Nucleic Acids Res 37:D211–D215. http://dx.doi.org/10.1093/nar/gkn785.

7. 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.

8. Xu J, Hendrix RW, Duda RL. 2004. Conserved translational frameshift in dsDNA bacteriophage tail assembly genes. Mol Cell 16:11–21. http://dx.doi.org/10.1016/j.molcel.2004.09.006.

9. Stoddard BL. 2014. Homing endonucleases from mobile group I introns: discovery to genome engineering. Mob DNA 5:7. http://dx.doi.org/10.1186/1759-8753-5-7.

10. Cech TR. 1990. Self-splicing of group I introns. Annu Rev Biochem 59:543–568. http://dx.doi.org/10.1146/annurev.bi.59.070190.002551.

11. Mikkonen M, Atalossava T. 1995. A group I intron in the terminase gene of Lactobacillus delbrueckii subsp. lactis phage LL-H. Microbiology 141:2183–2190. http://dx.doi.org/10.1099/13500872-141-9-2183.

12. Serwer P, Hayes SJ, Zaman S, Lieman K, Rolando M, Hardies SC. 2004. Improved isolation of undersampled bacteriophages: finding of distant terminase genes. Virology 329:412–424. http://dx.doi.org/10.1016/j.virol.2004.08.021.

13. Schuch R, Pelzek AJ, Fazzini MM, Nelson DC, Fischetti VA. 2014. Complete genome sequence of Bacillus cereus sensu lato bacteriophage Bcp1. Genome Announc 2(3):e00334-14. http://dx.doi.org/10.1128/genomeA.00334-14.

14. Maroun JW, Whitcher KJ, Chamakura KR, Kuty Everett GF. 2013. Complete genome of Bacillus thuringiensis myophage Spock. Genome Announc 1(6):e00334-14:e00863-13. http://dx.doi.org/10.1128/genomeA.00863-13.

15. Stewart CR, Casjens SR, Cresawn SG, Houtz JM, Smith AL, Ford ME, Peebles CL, Hatfull GF, Hendrix RW, Huang WM, Pedulla ML. 2009. The genome of Bacillus subtilis bacteriophage SPO1. J Mol Biol 388:48–70. http://dx.doi.org/10.1016/j.jmb.2009.03.009.

16. Wang IN, Smith DL, Young R. 2000. Holins: the protein clocks of bacteriophage infections. Annu Rev Microbiol 54:799–823. http://dx.doi.org/10.1146/annurev.micro.54.1.799.

17. Burton BM, Marquis KA, Sullivan NL, Rapoport TA, Rudner DZ. 2007. The ATPase SpoIIIE transports DNA across fused septal membranes during sporulation in Bacillus subtilis. Cell 131:1301–1312. http://dx.doi.org/10.1016/j.cell.2007.11.009.