



## Complete Genome Sequence of Bacillus megaterium Podophage Pavlov

Roberto W. Burgos, Scott J. Mash, Jesse L. Cahill, Eric S. Rasche, Gabriel F. Kuty Everett

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

*Bacillus megaterium* is a large spore-forming bacterium found widely in the environment. Phages infecting *B. megaterium* can be used as genetic tools to expand the array of uses of *B. megaterium* in research and industry. Here, we present the complete genome of Pavlov, a podophage infecting *B. megaterium*.

Received 20 July 2015 Accepted 24 July 2015 Published 3 September 2015

Citation Burgos RW, Mash SJ, Cahill JL, Rasche ES, Kuty Everett GF. 2015. Complete genome sequence of *Bacillus megaterium* podophage Pavlov. Genome Announc 3(5): e00993-15. doi:10.1128/genomeA.00993-15.

**Copyright** © 2015 Burgos et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license. Address correspondence to Gabriel F. Kuty Everett, cpt@tamu.edu.

**B***acillus megaterium* is a well-studied model organism in the field of microbiology. Due to its large size, secretion ability, and stable plasmid replication system, it has become a workhorse for recombinant protein production in the biotechnology industry (1). Recently, the systems metabolic engineering of *B. megaterium* has gained interest (2). Bacteriophages have a broad array of applications (3) and may play a role in developing the full potential of industrial *B. megaterium* strains. To that end, here we describe the novel *B. megaterium* podophage Pavlov.

Bacteriophage Pavlov was isolated from a soil sample collected in College Station, Texas, USA, based on its ability to grow on the asporogenic strain B. megaterium KM (ATCC 13632). 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). Qualitycontrolled, trimmed reads were assembled to a single contig of circular assembly at 25.4-fold coverage using SPAdes version 3.5.0. The contig was confirmed to be complete by PCR using primers that face the upstream and downstream ends of the contig. Products from the PCR amplification of the junctions of concatemeric molecules were sequenced by Sanger sequencing (Eton Bioscience, San Diego, CA, USA). 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/). The morphology of Pavlov was determined using transmission electron microscopy performed at the Texas A&M University Microscopy and Imaging Center.

Pavlov is a podophage with a 40-kb genome, a coding density of 96.2%, and a G+C content of 40.6%. Genome analysis and annotation shows 50 coding sequences, of which 22 have a predicted function by BLASTp and InterPro Scan (5, 6). Pavlov shares 89.0, 91.9, 91.0, 87.1, and 67.6% nucleotide sequence identity with recently described *B. megaterium* podophages Page (NCBI reference sequence NC\_022764) (7), Pony (NCBI reference sequence NC\_022770) (8), Pookie (GenBank accession number KM236248) (9), Palmer (KP411017) (10), and Pascal (KM236247) (11) as determined by Emboss Stretcher (12). Pavlov is predicted to use a *pac*type head-full DNA packaging mechanism and has been opened to the *terS* gene for annotation purposes (13). Host range studies show that Pavlov also infects the industrial *B. megaterium* strains PV361 and DSM 337 (1).

Replication, biosynthesis, packaging, transcriptional regulation, morphogenesis, lysis genes, and an HNH-homing endonuclease were identified in Pavlov. Three DNA-binding proteins, presumably transcriptional regulators, were found to contain lambda Cro/CI-type helix-turn-helix domains. Additionally, the presence of a plasmid replication/relaxation protein suggests that Pavlov may be a temperate phage. Like other phages of this group, Pavlov encodes an FtsK/SpoIIIE homolog. In Gram-positive bacteria, SpoIIIE is an ATPase that translocates DNA across the septal membrane of the sporulating mother cell into the forespore, although its role in the phage life cycle is unknown (14). Pavlov encodes a SigF-like sporulation-related sigma factor, indicating that the phage might influence the vegetative growth and sporulation of the host (15).

**Nucleotide sequence accession number.** The genome sequence of phage Pavlov was contributed to GenBank under the accession number KT001911.

## 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

- Vary PS, Biedendieck R, Fuerch T, Meinhardt F, Rohde M, Deckwer WD, Jahn D. 2007. *Bacillus megaterium*—from simple soil bacterium to industrial protein production host. Appl Microbiol Biotechnol 76: 957–967. http://dx.doi.org/10.1007/s00253-007-1089-3.
- Korneli C, David F, Biedendieck R, Jahn D, Wittmann C. 2013. Getting the big beast to work—systems biotechnology of *Bacillus megaterium* for novel high-value proteins. J Biotechnol 163:87–96. http://dx.doi.org/ 10.1016/j.jbiotec.2012.06.018.
- 3. Clark JR, March JB. 2006. Bacteriophages and biotechnology: vaccines,

gene therapy and antibacterials. Trends Biotechnol 24:212–218. http://dx.doi.org/10.1016/j.tibtech.2006.03.003.

- 4. Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a selftraining 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.
- 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.
- 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 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.
- 7. Lopez MS, Hodde MK, Chamakura KR, Kuty Everett GF. 2014. Complete genome of *Bacillus megaterium* podophage Page. Genome Announc 2(2):e00332-14. http://dx.doi.org/10.1128/genomeA.00332-14.
- Khatemi BE, Chung On CC, Chamakura KR, Kuty Everett GF. 2013. Complete genome of *Bacillus megaterium* podophage Pony. Genome Announc 1(6):e00860-13. http://dx.doi.org/10.1128/genomeA.00860-13.
- 9. Ladzekpo TN, DeCrescenzo AJ, Hernandez AC, Kuty Everett GF. 2015.

Complete genome of *Bacillus megaterium* podophage Pookie. Genome Announc 3(1):e01432-14. http://dx.doi.org/10.1128/genomeA.01432-14.

- Hargrove EC, Lopez MS, Hernandez AC, Kuty Everett GF. 2015. Complete genome sequence of *Bacillus megaterium* podophage Palmer. Genome Announc 3(3):e00358-15. http://dx.doi.org/10.1128/genomeA.00358-15.
- Snowden JD, Vega Gonzalez AE, Maroun JW, Hernandez AC, Kuty Everett GF. 2015. Complete genome of *Bacillus megaterium* podophage Pascal. Genome Announc 3(1):e01429-14. http://dx.doi.org/10.1128/ genomeA.01429-14.
- 12. 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.
- Casjens SR, Gilcrease EB. 2009. Determining DNA packaging strategy by analysis of the termini of the chromosomes in tailed-bacteriophage virions. Methods Mol Biol 502:91–111. http://dx.doi.org/10.1007/978-1 -60327-565-1\_7.
- 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.
- Yuan Y, Gao M, Wu D, Liu P, Wu Y. 2012. Genome characteristics of a novel phage from *Bacillus thuringiensis* showing high similarity with phage from *Bacillus cereus*. PLoS One 7:e37557. http://dx.doi.org/10.1371/ journal.pone.0037557.