



Genome Sequence of Mycobacteriophage Mindy

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Mycobacteriophage Mindy is a newly isolated phage of *Mycobacterium smegmatis*, recovered from a soil sample in Pittsburgh, Pennsylvania, USA. Mindy has a genome length of 75,796 bp, encodes 147 predicted proteins and two tRNAs, and is closely related to mycobacteriophages in cluster E.

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Bacteriophages, the most abundant biological entities in the biosphere, are viruses that infect bacterial hosts (1). Phages genetically are highly diverse and have therapeutic potential for controlling bacterial pathogens (2). A large collection of mycobacteriophages—phages that infect mycobacterial hosts—offers insights into phage evolution and provides a toolbox for genetic manipulation of *Mycobacterium tuberculosis* (3). The Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program involves large numbers of students at multiple institutions to gain research experience through phage isolation and genomic analysis (4).

Mycobacteriophage Mindy was isolated by direct plating of a soil sample from Pittsburgh, Pennsylvania, and DNA was isolated following plaque purification and phage amplification. The genome was sequenced on an Illumina MiSeq platform using140-bp single-end reads. Reads were assembled using Newbler into a single major contig with 4,365-fold coverage. The Mindy genome is 75,796 bp long with 9-bp 3' single-stranded DNA extensions with the sequence 5'-CGCTTGTCA. The genome has a G+C content of 63.0%.

GeneMark and Glimmer were used to generate a preliminary autoannotation—using heuristic and *Mycobacterium smegmatis* models—which was then revised following manual inspection. A total of 147 predicted protein-coding genes were identified and two tRNA genes were identified using Aragorn and tRNAscan-SE. Gene functions were predicted using BLASTp, HHpred, and domain searches in the program Phamerator (5) and predicted functions were assigned to 43 of the 147 predicted genes.

Mycobacteriophage Mindy is closely related to phages Cjw1, 244, Kostya, SirDuracell, Porky, and Rakim and joins them in cluster E. The most closely related phage is Henry (6), with which it shares 99% nucleotide identity across a span of 98% of its genome. As in other cluster E phages, the genome architecture is such that there are two large blocks of rightward-transcribed genes and two smaller blocks of leftward-transcribed genes, with several interruptions of 1 to 2 genes. The terminase large subunit (gp9) contains an intein and the capsid subunit (gp13) is predicted to assemble similarly to the HK97 capsid (7). The Mindy genome

includes two related adjacent genes 94 and 95 (51% amino acid identity) and may be a precursor to those phages containing homologues of only one of these (e.g., Henry 93 and Toto 93). Two Mindy gene products are predicted to have interesting DNA binding properties: gp75, which is predicted to be structurally related to DNA processing protein A (8), and gp57, which is structurally related to the MfpA protein of *M. tuberculosis* (Rv3361c), which confers resistance to fluoroquinolones by mimicking the structure of dsDNA and binding to DNA gyrase (9). Mindy gp56 is predicted to be expressed during early lytic growth, and it is unclear whether it binds to the host MfpA protein or to a different target through DNA mimicry.

Nucleotide sequence accession number. The Mindy genome sequence is accessible from GenBank under the accession number KR080204.

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REFERENCES

- Hatfull GF, Hendrix RW. 2011. Bacteriophages and their genomes. Curr Opin Virol 1:298–303. http://dx.doi.org/10.1016/j.coviro.2011.06.009.
- Nobrega FL, Costa AR, Kluskens LD, Azeredo J. 2015. Revisiting phage therapy: new applications for old resources. Trends Microbiol 23:185–191 http://dx.doi.org/10.1016/j.tim.2015.01.006.
- 3. Hatfull GF. 2012. The secret lives of mycobacteriophages. Adv Virus Res 82:179–288. http://dx.doi.org/10.1016/B978-0-12-394621-8.00015-7.
- 4. Jordan TC, Burnett SH, Carson S, Caruso SM, Clase K, DeJong RJ, Dennehy JJ, Denver DR, Dunbar D, Elgin SCR, Findley AM, Gissend-anner CR, Golebiewska UP, Guild N, Hartzog GA, Grillo WH, Hollowell GP, Hughes LE, Johnson A, King RA, Lewis LO, Li W, Rosenzweig F, Rubin MR, Saha MS, Sandoz J, Shaffer CD, Taylor B, Temple L, Vazquez E, Ware VC, Barker LP, Bradley KW, Jacobs-Sera D, Pope WH, Russell DA, Cresawn SG, Lopatto D, Bailey CP, Hatfull GF. 2014. A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. mBio 5:e01051-01013. http://dx.doi.org/ 10.1128/mBio.01051-13.
- Cresawn SG, Bogel M, Day N, Jacobs-Sera D, Hendrix RW, Hatfull GF. 2011. Phamerator: a bioinformatic tool for comparative bacteriophage

genomics. BMC Bioinformatics 12:395. http://dx.doi.org/10.1186/1471 -2105-12-395.

- Hatfull GF, Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science Program, KwaZulu-Natal Research Institute for Tuberculosis and HIV Mycobacterial Genetics Course Students, Phage Hunters Integrating Research and Education Program. 2012. Complete genome sequences of 138 mycobacteriophages. J Virol 86: 2382–2384. http://dx.doi.org/10.1128/JVI.06870-11.
- 7. Helgstrand C, Wikoff WR, Duda RL, Hendrix RW, Johnson JE, Liljas L. 2003. The refined structure of a protein catenane: the HK97 bacteriophage

capsid at 3.44 Å resolution. J Mol Biol 334:885-899. http://dx.doi.org/ 10.1016/j.jmb.2003.09.035.

- Wang W, Ding J, Zhang Y, Hu Y, Wang DC. 2014. Structural insights into the unique single-stranded DNA-binding mode of *Helicobacter pylori* DprA. Nucleic Acids Res 42:3478–3491. http://dx.doi.org/10.1093/nar/ gkt1334.
- Hegde SS, Vetting MW, Roderick SL, Mitchenall LA, Maxwell A, Takiff HE, Blanchard JS. 2005. A fluoroquinolone resistance protein from *Myco-bacterium* tuberculosis that mimics DNA. Science 308:1480–1483. http:// dx.doi.org/10.1126/science.1110699.