



Complete Genome Sequence of *Stenotrophomonas* Phage Mendera

Kameron D. Garza, a Heather Newkirk, a Russell Moreland, a Carlos F. Gonzalez, a Mei Liu, a 💿 Jolene Ramsey, a Justin Leavitta

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

ABSTRACT Stenotrophomonas maltophilia is an emerging opportunistic human pathogen. In this report, we describe the isolation and genomic annotation of the *S. maltophilia*-infecting bacteriophage Mendera. A myophage of 159,961 base pairs, Mendera is T4-like and related most closely to *Stenotrophomonas* phage IME-SM1.

Stenotrophomonas maltophilia is a rod-shaped, Gram-negative, nonfermenting, and motile obligate aerobe. Ubiquitous in its distribution, *S. maltophilia* resides in bodies of water, in sewage, on plants, and in the respiratory tract of humans as an opportunistic pathogen (1). As *S. maltophilia* is an opportunist, infection caused by the bacterium is a major problem for cystic fibrosis patients (2). In this report, we describe the isolation and genomic annotation of the *S. maltophilia*-infecting bacteriophage Mendera.

The source for Mendera was filtered (0.2 μ m) wastewater collected in Navasota, TX. For isolation, the host S. maltophilia (ATCC 17807) was grown aerobically at 30°C in nutrient broth or agar (BD), and phage was propagated by the soft-agar overlay method (3). Mendera myophage morphology was identified via negative-stain transmission electron microscopy with 2% (wt/vol) uranyl acetate at the Texas A&M Microscopy and Imaging Center (4). Phage genomic DNA was purified with the Promega Wizard DNA clean-up system modified according to the shotgun library preparation protocol (5). Libraries were prepared with the Illumina TruSeq Nano low-throughput kit and sequenced via Illumina MiSeq with paired-end 250-bp reads using V2 500-cycle chemistry. The 222,492 total sequence reads were quality controlled with FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc), trimmed using the FastX toolkit v0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/), and assembled into a single raw contig at 11.6-fold coverage using SPAdes v3.5.0 at default parameters (6). Contig accuracy and completeness were confirmed by PCR (forward primer, 5'-GTCACCGCGA CTACGATAAG-3'; reverse primer, 5'-GAACGACAGCGAGCATACA-3') off the contig ends and Sanger sequencing of the product. Possible protein-coding genes were annotated using GLIMMER v3.0 and MetaGeneAnnotator v1.0, and tRNA genes were annotated from ARAGORN v2.36 (7-9). Rho-independent termination sites were annotated using TransTermHP v2.09 (10). To predict protein functions, we used BLAST v2.2.31 sequence similarity searches conducted against the NCBI nonredundant, UniProtKB Swiss-Prot, and TrEMBL databases with a 0.001 maximum expectation value and conserved domain searches via InterProScan v5.33-72 (11-13). ProgressiveMauve v2.4.0 was used to calculate genome-wide DNA sequence similarity (14). PhageTerm was used to predict genomic termini (15). The bioinformatics tools were accessed through the Center for Phage Technology Galaxy and Web Apollo instances hosted at https://cpt.tamu.edu/ galaxy-pub/ (16, 17). Unless otherwise stated, all tools were executed using default parameters.

The 159,961-bp double-stranded DNA genome of myophage Mendera has a 97% protein-coding density and a G+C content of 54%, which is lower than the host G+C

Citation Garza KD, Newkirk H, Moreland R, Gonzalez CF, Liu M, Ramsey J, Leavitt J. 2020. Complete genome sequence of *Stenotrophomonas* phage Mendera. Microbiol Resour Announc 9:e01411-19. https://doi.org/ 10.1128/MRA.01411-19.

Editor John J. Dennehy, Queens College Copyright © 2020 Garza et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Justin Leavitt, justincleavitt@gmail.com.

Received 12 November 2019 Accepted 20 November 2019 Published 2 January 2020 content of 66.8% (18). In total, 287 protein-coding genes and 23 tRNA genes were predicted. Mendera is related most closely to *Stenotrophomonas* phage IME-SM1, with 198 similar proteins and 92.85% nucleotide identity (GenBank accession no. KR560069). Consistent with its genomic organization and myophage morphology being T4-like, Mendera is predicted to use a headful packaging mechanism.

Data availability. The genome sequence and associated data for phage Mendera were deposited under GenBank accession no. MN098328, BioProject no. PRJNA222858, SRA no. SRR8893604, and BioSample no. SAMN11414489.

ACKNOWLEDGMENTS

This work was supported by funding from the National Science Foundation (award DBI-1565146) and the Citrus Research and Development Foundation (project C726) to C.F.G. Additional support came from the Center for Phage Technology (CPT), an Initial University Multidisciplinary Research Initiative supported by Texas A&M University and Texas AgriLife, and from the Texas A&M University 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 Bacteriophage Genomics, an undergraduate course at Texas A&M University.

REFERENCES

- Brooke JS. 2012. Stenotrophomonas maltophilia: an emerging global opportunistic pathogen. Clin Microbiol Rev 25:2–41. https://doi.org/10 .1128/CMR.00019-11.
- Lipuma JJ. 2010. The changing microbial epidemiology in cystic fibrosis. Clin Microbiol Rev 23:299–323. https://doi.org/10.1128/CMR.00068-09.
- 3. Adams MH. 1959. Bacteriophages. Interscience Publishers, Inc., New York. NY.
- Valentine RC, Shapiro BM, Stadtman ER. 1968. Regulation of glutamine synthetase. XII. Electron microscopy of the enzyme from Escherichia coli. Biochemistry 7:2143–2152. https://doi.org/10.1021/bi00846a017.
- Summer EJ. 2009. Preparation of a phage DNA fragment library for whole genome shotgun sequencing. Methods Mol Biol 502:27–46. https://doi.org/10.1007/978-1-60327-565-1_4.
- 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. https://doi.org/10.1089/cmb.2012.0021.
- Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res 27: 4636–4641. https://doi.org/10.1093/nar/27.23.4636.
- Noguchi H, Taniguchi T, Itoh T. 2008. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. DNA Res 15:387–396. https://doi.org/10.1093/dnares/dsn027.
- Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16. https://doi.org/10.1093/nar/gkh152.
- Kingsford CL, Ayanbule K, Salzberg SL. 2007. Rapid, accurate, computational discovery of Rho-independent transcription terminators illuminates their relationship to DNA uptake. Genome Biol 8:R22. https://doi .org/10.1186/gb-2007-8-2-r22.

- Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421.
- Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, Pesseat S, Quinn AF, Sangrador-Vegas A, Scheremetjew M, Yong SY, Lopez R, Hunter S. 2014. InterProScan 5: genome-scale protein function classification. Bioinformatics 30: 1236–1240. https://doi.org/10.1093/bioinformatics/btu031.
- The UniProt Consortium. 2019. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 47:D506–D515. https://doi.org/10.1093/ nar/gky1049.
- Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. https://doi.org/10.1371/journal.pone.0011147.
- Garneau JR, Depardieu F, Fortier LC, Bikard D, Monot M. 2017. PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data. Sci Rep 7:8292. https://doi.org/10.1038/s41598-017-07910-5.
- Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Cech M, Chilton J, Clements D, Coraor N, Gruning BA, Guerler A, Hillman-Jackson J, Hiltemann S, Jalili V, Rasche H, Soranzo N, Goecks J, Taylor J, Nekrutenko A, Blankenberg D. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res 46:W537–W544. https://doi.org/10.1093/nar/gky379.
- Lee E, Helt GA, Reese JT, Munoz-Torres MC, Childers CP, Buels RM, Stein L, Holmes IH, Elsik CG, Lewis SE. 2013. Web Apollo: a Web-based genomic annotation editing platform. Genome Biol 14:R93. https://doi .org/10.1186/gb-2013-14-8-r93.
- Lira F, Berg G, Martinez JL. 2017. Double-face meets the bacterial world: the opportunistic pathogen Stenotrophomonas maltophilia. Front Microbiol 8:2190. https://doi.org/10.3389/fmicb.2017.02190.