



Complete Sequence and Annotation of the *Mycoplasma phocidae* Strain 105^T Genome

Salvatore Frasca, Jr.,^a Gerald F. Kutish,^b Dina L. Michaels,^c Daniel R. Brown^c

^aDepartment of Comparative, Diagnostic and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

^bDepartment of Pathobiology and Veterinary Science and Center of Excellence for Vaccine Research, University of Connecticut, Storrs, Connecticut, USA

^cDepartment of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

ABSTRACT The genome of *Mycoplasma phocidae* strain 105^T was analyzed in order to improve our understanding of its role in epidemic marine mammal mortalities. It was found to encode a suite of immunosuppressors that may enable evasion of host defenses and modulate susceptibility to viral coinfections or their severity in seals.

Mycoplasma phocidae strain 105^T was first isolated from the lungs of one of more than 400 harbor seals (*Phoca vitulina*) that died along the U.S. New England coastline during a mass mortality event in 1980. Epidemic seal deaths occurring in the North Atlantic in 1988 and 2002 were attributed primarily to phocine distemper virus or H7N7 influenza virus, but coinfection with *M. phocidae* was common (1–3). In order to improve our ability to explain the role of *M. phocidae* in marine mammal mortalities and the implications of such events for human and ocean health, we sequenced and annotated the genome of a lyophilized specimen of strain 105^T acquired by the Mollicutes Collection (World Data Centre for Microorganisms Collection 858) from the ATCC (catalog number 33657) in 1990. The DNA was extracted using an Easy-DNA genomic DNA purification kit (catalog number K180001; Thermo Fisher Scientific). Sequencing libraries were constructed using the NEBNext UltraTMII DNA library prep kit (catalog number E76455; New England Biolabs) and dual-index NEBNext Multiplex Oligos (catalog number E76005; New England Biolabs). Illumina MiSeq 2 × 300-bp paired-end sequencing yielded approximately 5 × 10⁷ Phred quality-filtered reads with a quality score greater than 30. The final assembly with coverage depth of more than 800× was achieved in May 2018 by using a combination of Ray v2.3.1, Unicycler v0.4.3, and Consed v29 finishing software (4–6) and then annotated with the RAST server and the NCBI Prokaryotic Genome Annotation Pipeline (7, 8), all with default settings.

The closed circular genome is 814,478 bp long, with a G+C content of 27.1 mol%, close to the 27.8 mol% estimated by buoyant density (3). Of 657 predicted open reading frames (ORFs), about two-thirds have assigned functions, and about half could be grouped in subsystems, including 40 structural RNAs, 181 proteins involved in nucleic acid metabolism, transcription, translation, or protein fate, 45 in amino acid, purine, pyrimidine, nucleoside, nucleotide, cofactor, prosthetic group, or carrier metabolism, 27 in carbohydrate metabolism, 9 in fatty acid or phospholipid metabolism, 11 in transport or binding processes, 5 in cell division, and 8 chaperones, elongation factors, or transcription regulators. A complete arginine dihydrolase pathway of ATP synthesis is present as expected for this member of the *Mycoplasma hominis* phylogenetic cluster (9). We interpret the presence of genes encoding cytosine 5-methyltransferase (CpG DNA methylase), adenine N-6 methyltransferase, and type II and III restriction-modification

Received 10 September 2018 **Accepted** 19 September 2018 **Published** 11 October 2018

Citation Frasca S, Jr, Kutish GF, Michaels DL, Brown DR. 2018. Complete sequence and annotation of the *Mycoplasma phocidae* strain 105^T genome. Microbiol Resour Announc 7:e01237-18. <https://doi.org/10.1128/MRA.01237-18>.

Editor Frank J. Stewart, Georgia Institute of Technology

Copyright © 2018 Frasca et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Daniel R. Brown, drbrown@ufl.edu.

system DNA methylases as evidence of the capacity for epigenetic modifications of the genome.

M. phocidae encodes several candidate virulence factors, including a family of putative surface antigens with about 60% amino acid sequence similarity over their proline-rich N-terminal region, to the pneumococcal complement evasion protein PspA (10); a family of proteins with about 40% sequence similarity to mycoplasmal protein M, which binds the light chain region of IgG to block antigen-specific binding (11); and a predicted immunoglobulin heavy chain-binding protein similar to the phocine host-specific IgG endoprotease IdeP of *Streptococcus phocae* (12) fused to the mycoplasmal lipoprotein LppA. It remains to be confirmed whether this suite of immunosuppressors constitutes a mechanism by which *M. phocidae* evades host defenses and predisposes susceptibility or modulates outcomes of influenza or distemper in seals.

Data availability. The *M. phocidae* 105^T genome sequence and annotation data have been deposited in GenBank under the accession number [CP029295](https://doi.org/10.1101/202001); the sequence described in this paper is the first version, CP029295.1. The raw data are available in the NCBI Sequence Read Archive under accession number [SRX4702894](https://doi.org/10.1101/202001).

ACKNOWLEDGMENTS

This work was supported by University of Florida faculty development funds (S.F.).

Library construction was performed by David Moraga at the Interdisciplinary Center for Biotechnology Research at the University of Florida. Thomas B. Waltzek and Kutchantran Subramaniam performed the Illumina sequencing and preliminary sequence assembly at the University of Florida Wildlife and Aquatic Veterinary Disease Laboratory.

We declare no conflicts of interest.

REFERENCES

- Geraci JR, St Aubin DJ, Barker IK, Webster RG, Hinshaw VS, Bean WJ, Ruhnke HL, Prescott JH, Early G, Baker AS, Madoff S, Schooley RT. 1982. Mass mortality of harbor seals: pneumonia associated with influenza A virus. *Science* 215:1129–1131. <https://doi.org/10.1126/science.7063847>.
- Müller G, Kaim U, Haas L, Greiser-Wilke I, Wohlsein P, Siebert U, Baumgärtner W. 2008. Phocine distemper virus: characterization of the morbillivirus causing the seal epizootic in northwestern Europe in 2002. *Arch Virol* 153:951–956. <https://doi.org/10.1007/s00705-008-0055-4>.
- Ruhnke HL, Madoff S. 1992. *Mycoplasma phocidae* sp. nov., isolated from harbor seals (*Phoca vitulina* L.). *Int J Syst Bacteriol* 42:211–214. <https://doi.org/10.1099/00207713-42-2-211>.
- Boisvert S, Laviolette F, Corbeil J. 2010. Ray: simultaneous assembly of reads from a mix of high-throughput sequencing technologies. *J Comput Biol* 17:1519–1533. <https://doi.org/10.1089/cmb.2009.0238>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Gordon D, Abajian C, Green P. 1998. Consed: a graphical tool for sequence finishing. *Genome Res* 8:195–202. <https://doi.org/10.1101/gr.8.3.195>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman K, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Ciufu S, Li W. 2013. Prokaryotic genome annotation pipeline, p 173–186. *In* Beck J, Benson D, Coleman J, Hoepfner M, Johnson M, Maglott M, Mizrahi I, Morris R, Ostell J, Pruitt K, Rubinstein W, Sayers E, Sirotkin K, Tatusova T (ed), *The NCBI handbook*, 2nd ed. NCBI, Bethesda, MD.
- Pettersson B, Tully JG, Bölske G, Johansson KE. 2000. Updated phylogenetic description of the *Mycoplasma hominis* cluster (Weisburg et al. 1989) based on 16S rDNA sequences. *Int J Syst Evol Microbiol* 50: 291–301. <https://doi.org/10.1099/00207713-50-1-291>.
- Jedrzejewski MJ. 2001. Pneumococcal virulence factors: structure and function. *Microbiol Mol Biol Rev* 65:187–207. <https://doi.org/10.1128/MMBR.65.2.187-207.2001>.
- Grover RK, Zhu X, Nieuwma T, Jones T, Boreo I, MacLeod AS, Mark A, Niessen S, Kim HJ, Kong L, Assad-Garcia N, Kwon K, Chesi M, Smider VV, Salomon DR, Jelinek DF, Kyle RA, Pyles RB, Glass JI, Ward AB, Wilson IA, Lerner RA. 2014. A structurally distinct human mycoplasma protein that generically blocks antigen-antibody union. *Science* 343:656–661. <https://doi.org/10.1126/science.1246135>.
- Rungelrath V, Wohlsein JC, Siebert U, Stott J, Prenger-Berninghoff E, von Pawel-Rammingen U, Valentin-Weigand P, Baums CG, Seele J. 2017. Identification of a novel host-specific IgG protease in *Streptococcus phocae* subsp. *phocae*. *Vet Microbiol* 201:42–48. <https://doi.org/10.1016/j.jvetmic.2017.01.009>.