Permanent Draft Genome Sequence of *Photorhabdus temperata* Strain Hm, an Entomopathogenic Bacterium Isolated from Nematodes

Shimaa Ghazal,a,b Erik Swanson,a Stephen Simpson,a Krystalynne Morris,a Feseha Abebe-Akele,a W. Kelley Thomas,a Kamal M. Khalil,b Louis S. Tisaa

University of New Hampshire, Durham, New Hampshire, USAa; Applied Microbial Genetics Laboratory, Genetics and Cytology Department, Genetic Engineering & Biotechnology Division, National Research Center, Dokki, Cairo, Egyptb

**ABSTRACT** *Photorhabdus temperata* strain Hm is an entomopathogenic bacterium that forms a symbiotic association with *Heterorhabditis* nematodes. Here, we report a 5.0-Mbp draft genome sequence for *P. temperata* strain Hm with a G+C content of 44.1% and containing 4,226 candidate protein-encoding genes.

Members of the genus *Photorhabdus* maintain two distinct lifestyles as insect pathogens and in a symbiotic relationship with the entomopathogenic *Heterorhabditis* nematodes (for reviews, see references 1–3). The *Heterorhabditis* nematodes carry a monoculture of *Photorhabdus* within the anterior region of the infective juvenile (IJ) nematode’s intestine (4, 5) and actively seek insect prey in the soil. The nematodes infect a wide range of insect hosts by entering through natural openings or by burrowing directly through the insect cuticle. Once inside the insect, the nematodes regurgitate the bacteria into the hemolymph (4). The bacteria kill the insect within 48 h by releasing highly virulent toxins (6–9). As the bacteria enter the stationary phase of their growth cycle, they secrete extracellular enzymes that aid in breaking down insect tissue, thereby providing nutrients for both the bacteria and the nematodes. The bacteria also generate essential growth factors for nematode growth and development. The growth and development of *Heterorhabditis* nematodes have an obligate requirement for their specific bacterial symbiont (10). The bacteria also release antibiotics to prevent secondary invaders and putrefaction of the insect carcass (11, 12). After several days of feeding, the nematodes and bacteria reassociate and leave in search of a new insect host.

Members of *Photorhabdus* are classified taxonomically into one of three species, *P. luminescens*, *P. temperata*, or *P. asymbiotica* (13–15). Several subspecies are recognized. Our understanding of these bacteria has been greatly enhanced by genome sequencing of the three established species, including that of *P. luminescens* TT01 (16), *P. asymbiotica* ATCC 43949 (17), *P. temperata* subsp. *khanii* NC19 (18), *P. temperata* Meg1 (19), *P. luminescens* BA1 (20), *P. luminescens* subsp. *laumondii* HP88 (21), *P. asymbiotica* Kingcliff (22), *P. temperata* subsp. *temperata* M121 (23), *P. luminescens* subsp. PB45.5 (24), and *P. asymbiotica* subsp. australis PB68.1 (24). Here, we present a draft genome sequence for *P. temperata* strain Hm, which was isolated from *Heterorhabditis* nematodes found in Georgia (10).

The draft genome sequence of *P. temperata* strain Hm was generated at the Hubbard Genome Center (University of New Hampshire, Durham, NH) using Illumina technology (25) techniques. A standard Illumina shotgun library was constructed and sequenced using the Illumina HiSeq2500 platform, which generated 15,461,198 reads (150-bp insert size) totaling 2,303.7 Mbp. The Illumina sequence data were trimmed by...
Prokaryotic Genome Annotation Pipeline (PGAP) and resulted in 4,226 candidate protein-encoding genes and 68 tRNA and 4 rRNA regions.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number MBJU00000000. The version described in this paper is MBIU01000000.

ACKNOWLEDGMENTS

This work was supported in part by USDA NIFA grant 2009-35302-05257 and by the College of Life Science and Agriculture at the University of New Hampshire-Durham. S.G. was supported by the Egyptian Cultural and Educational Bureau, Washington, DC, USA.

Sequencing was performed on an Illumina HiSeq2500 instrument purchased with NSF MRI grant DBI-122961 to W.K.T.

REFERENCES

1. Goodrich-Blair H, Clarke DJ. 2007. Mutualism and pathogenesis in Xenorhabdus and Photorhabdus: two roads to the same destination. Mol Microbiol 64:260–268. https://doi.org/10.1111/j.1365-2958.2007.05671.x.

2. Clarke DJ. 2008. Photorhabdus: a model for the analysis of pathogenicity and mutualism. Cell Microbiol 10:2159–2167. https://doi.org/10.1111/j.1462-5822.2008.01209.x.

3. Waterfield NR, Ciche T, Clarke D. 2009. Photorhabdus and a host of hosts. Annu Rev Microbiol 63:557–574. https://doi.org/10.1146/annurev.micro.091208.073507.

4. Ciche TA, Ensign JC. 2003. For the insect pathogen Photorhabdus luminescens, which end of a nematode is out? Appl Environ Microbiol 69:1890–1897. https://doi.org/10.1128/AEM.69.4.1890-1897.2003.

5. Endo BY, Nickle WR. 1991. Ultrastructure of the intestinal epithelium, lumen, and associated bacteria in Heterorhabditis-Bacteriophora. J Helminthol Soc Wash 58:202–212.

6. Bowen D, Rocheleau TA, Blackburn M, Andreev O, Golubeva E, Bhartia R, ffrench-Constant RH, Bowen D. 1998. Purification and characterization of a high-molecular-weight insecticidal protein complex produced by the entomopathogenic bacterium Photorhabdus luminescens. Appl Environ Microbiol 64:3029–3035.

7. Bowen D, Ensign JC. 1998. Purification and characterization of a high-molecular-weight insecticidal protein complex produced by the entomopathogenic bacterium Photorhabdus luminescens. Appl Environ Microbiol 64:3029–3035.

8. ffrench-Constant RH, Bowen D. 1999. Photorhabdus toxins: novel biological insecticides. Curr Opin Microbiol 2:284–288. https://doi.org/10.1016/S0928-8947(99)00049-6.

9. ffrench-Constant RH, Bowen DJ. 2000. Novel insecticidal toxins from nematode-symbiotic bacteria. Cell Mol Life Sci 57:828–833. https://doi.org/10.1007/s000180050044.

10. Thomas GM, Poinar GO. 1979. Xenorhabdus gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family Enterobacteriaceae. Int J Syst Bacteriol 29:352–360. https://doi.org/10.1099/00221287-29-1-352.

11. Akhurst RJ. 1982. Antibiotic activity of Xenorhabdus spp., bacteria symbiotically associated with insect pathogenic nematodes of the families Heterorhabditidae and Steinernematidae. J Gen Microbiol 128:3061–3065. https://doi.org/10.1099/00221287-128-12-3061.

12. Richardson WH, Schmidt TM, Nealon KH. 1988. Identification of an anthraquinone pigment and a hydroxystilbene antibiotic from Xenorhabdus luminescens. Appl Environ Microbiol 54:1602–1605.

13. Fischer-Le Saux M, Viallard V, Brunel B, Normand P, Boemare NE. 1999. Polyphasic classification of the genus Photorhabdus and proposal of new taxa: P. luminescens subsp. luminescens subsp. nov., P. luminescens subsp. akhurstii subsp. nov., P. luminescens subsp. laumondii subsp. nov., P. temperata subsp. nov., P. temperata subsp. temperata subsp. nov. and P. asymbiotica sp. nov. Int J Syst Bacteriol 49:1645–1656. https://doi.org/10.1099/00221287-49-4-1645.

14. Tailliez P, Pagès S, Ginibre N, Boemare N. 2006. New insight into diversity in the genus Xenorhabdus, including the description of ten novel species. Int J Syst Evol Microbiol 56:2805–2818. https://doi.org/10.1099/ijs.0.64287-0.

15. Tousi S, Blight M, Joasa S, Pimenta AD. 2006. From insects to human hosts: identification of major genomic differences between entomopathogenic strains of Photorhabdus and the emerging human pathogen Photobacterium asymbiotica. Int J Med Microbiol 296:521–530. https://doi.org/10.1016/j.ijmm.2006.04.004.

16. Duchaud E, Rusniok C, Frangeul L, Buchrieser C, Givaudan A, Taourit S, Bocs S, Boursaux-Eude C, Chandler M, Charles JF, Dassa E, Derose R, Derzelle S, Freyssinet G, Gaudriault S, Médiagne C, Lanois A, Powell K, Siguier P, Vincent R, Wingate V, Zouine M, Glaser P, Boemare N, Danchin A, Kunst F. 2003. The genome sequence of the entomopathogenic bacterium Photorhabdus luminescens. Nat Biotechnol 21:1307–1313. https://doi.org/10.1038/nbt886.

17. Wilkinson P, Waterfield NR, Crossman L, Corton C, Sanchez-Contreras M, Vlisidou I, Barron A, Bignell A, Clark L, Ormond D, Mayho M, Bason N, Smith F, Simmonds M, Churcher C, Harris D, Thompson NR, Quail M, Parkhill J, ffrench-Constant RH. 2009. Comparative genomics of the emerging human pathogen Photobacterium asymbiotica with the insect pathogen Photorhabdus luminescens. BMC Genomics 10:302. https://doi.org/10.1186/1471-2164-10-302.

18. Hust S, Rowedder H, Michaels B, Bullock H, Jackobeck R, Abebe-Akele F, Durakovic U, Gately J, Janicki E, Tisa LS. 2015. Elucidation of the Photobacterium temperata genome and generation of a transposon mutant library to identify motility mutants altered in pathogenesis. J Bacteriol 197:2201–2216. https://doi.org/10.1128/JB.00197-15.

19. Hust SG, Ghazal S, Morris K, Abebe-Akele F, Thomas WK, Badr UM, Hussein MA, Abou-Zaied MA, Khalil KM, Tisa LS. 2014. Draft genome sequence of Photobacterium temperata strain Meg1, an entomopathogenic bacterium isolated from Heterorhabditis megidis nematodes. Genome Announc 2(6):e01273-14. https://doi.org/10.1128/genomeA.01273-14.

20. Ghazal S, Hust SG, Morris K, Abebe-Akele F, Thomas WK, Badr UM, Hussein MA, Abou-Zaied MA, Khalil KM, Tisa LS. 2014. Draft genome sequence of Photobacterium luminescens strain BA1, an entomopathogenic bacterium isolated from nematodes found in Egypt. Genoscope 22(2):e00396-14. https://doi.org/10.1128/genomeA.00396-14.

21. Ghazal S, Oshone R, Simpson S, Morris K, Abebe-Akele F, Thomas WK, Khalil KM, Tisa LS. 2016. Draft genome sequence of Photobacterium luminescens subsp. laumondii HP88, an entomopathogenic bacterium isolated from nematodes. Genome Announce 4(2):e00154-16. https://doi.org/10.1128/genomeA.00154-16.

22. Wilkinson P, Paszkiewicz K, Moorhouse A, Szubert JM, Beatson S, Gerrard J, Waterfield NR, ffrench-Constant RH. 2010. New plasmids and putative genes of Xenorhabdus appl. Microbiol. 2010 Announc 2(2):e00396-14.
of *Photorhabdus asymbiotica*. FEMS Microbiol Lett 309:136–143. https://doi.org/10.1111/j.1574-6968.2010.02030.x.

23. Park GS, Khan AR, Hong SJ, Jang EK, Ullah I, Jung BK, Choi J, Yoo NK, Park KJ, Shin JH. 2013. Draft genome sequence of entomopathogenic bacterium *Photorhabdus temperata* strain M1021, isolated from nematodes. Genome Announc 1(5):e00154-16. https://doi.org/10.1128/genomeA.00747-13.

24. Tobias NJ, Mishra B, Gupta DK, Sharma R, Thines M, Stinear TP, Bode HB. 2016. Genome comparisons provide insights into the role of secondary metabolites in the pathogenic phase of the *Photorhabdus* life cycle. BMC Genomics 17:537. https://doi.org/10.1186/s12864-016-2862-4.

25. Bennett S. 2004. Solexa Ltd. Pharmacogenomics 5:433–438. https://doi.org/10.1517/14622416.5.4.433.

26. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

27. Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Daza R, Williams L, Nicol R, Gnirke A, Nusbaum C, Lander ES, Jaffe DB. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci USA 108:1513–1518. https://doi.org/10.1073/pnas.1017351108.