PROKARYOTES



Draft Genome Sequence of *Photorhabdus luminescens* HIM3 Isolated from an Entomopathogenic Nematode in Agricultural Soils

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ABSTRACT In this work, we report the draft genome sequence of *Photorhabdus luminescens* strain HIM3, a symbiotic bacterium associated with the entomopathogenic nematode *Heterorhabditis indica* MOR03, isolated from soil sugarcane in Yautepec, Morelos, Mexico. These bacteria have a G+C content of 42.6% and genome size of 5.47 Mb.

The bacterium *Photorhabdus luminescens* is a gammaproteobacterium, is Gram negative, and is a member of the *Enterobacteriaceae* family; it is highly lethal for a wide spectrum of insects and maintains a symbiotic relationship with nematodes of the genus *Heterorhabditis* (1). The nematode carries the bacterium in the intestine during the juvenile infective stage (IJ), which penetrates an insect and regurgitates the symbionts in the hemolymph; these bacteria kill the insect in 24 to 48 h (2, 3). Therefore, *P. luminescens* is a good model for understanding the genetic bases involved in mutualism with nematodes and pathogenicity against insects, as well as being a source of insecticidal toxins (4, 5).

We sequenced the genome of the bacterium P. luminescens strain HIM3, isolated from the entomopathogenic nematode Heterorhabditis indica MOR03, recovered from soil cultivated with sugarcane in Yautepec, Morelos, Mexico (18°55'16.9"N, 99°02'24.0"W). The bacteria were grown in LB broth and incubated for 12 h at 30°C with shaking at 250 rpm; genomic DNA was extracted with the ZR fungal/bacterial miniprep kit (Zymo Research), and 5 μ g of genomic DNA was sequenced using the Illumina HiSeq platform (2 imes 300 bp paired-end reads). A total of 814,835 reads were obtained, and quality trimming and error correction of the reads were performed using a DynamicTrim (SolexaQA++) (6) Perl script. Reads were assembled using SPAdes version 3.5.0 (7), and the draft genome resulted in 91 contigs with a total length of 5,472,253 bp. The largest contig was 706,123 bp, the N_{50} value was 232,526 bp, and the L_{50} was reached in 8 contigs. The genome sequence of P. luminescens subsp. laumondii TTO1 was used as a reference. Automated annotation was performed with Rapid Annotations using Subsystems Technology (RAST) (8). The genome of P. luminescens HIM3 has a G+C content of 42.6% and includes 4,659 coding sequences; using RNAmmer and Aragorn, 10 rRNAs (5S, 16S, and 23S) and 70 tRNAs, respectively, were obtained (9, 10). Based on the analysis of concatenated sequences (gyrA, gyrB, dnaN, and 16S and 23S rRNA genes) P. luminescens HIM3 is closely related to P. luminescens DSM 3368 (GenBank accession no. NZ_JXSK00000000). The genome of P. luminescens HIM3 contains genes encoding the type III secretion system (T3SS) responsible for the injection of virulence proteins

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Address correspondence to Edgar Dantán-González, edantan@uaem.mx. into the cytoplasm of eukaryotic cells, and it contains the gene coding for YopT, an effector protein that inhibits phagocytosis in insect macrophages (11). Interestingly, besides pathogenesis, it is suggested that T3SS is involved in other symbiotic relationships, such as mutualism and parasitism (12). We also found genes encoding the repeats-in toxin (RTX)-like toxins, including PrtA, a metalloprotease secreted by the type I secretion system (T1SS), which has been proposed to be involved in degrading insect tissues, which are food sources of nematode and bacteria (13). Also, genes coding for multiple type II toxin-antitoxin modules and colicin-like bacteriocins that prevent the growth of nonsymbiotic bacteria and insect putrefaction were found. The presence of plasmids was not found using PlasmidFinder 1.3 (14). We found genes necessary for the efficient uptake of iron, an important factor for pathogenicity and mutualism (15). Finally, large numbers of genes with probable insecticidal activity were found in the genome of *P. luminescens* HIM3.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. MYFJ00000000. The version described in this paper is version MYFJ01000000.

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REFERENCES

- Forst S, Dowds B, Boemare N, Stackebrandt E. 1997. Xenorhabdus and Photorhabdus spp.: bugs that kill bugs. Annu Rev Microbiol 51:47–72. https://doi.org/10.1146/annurev.micro.51.1.47.
- 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.
- Ciche TA, Kim KS, Kaufmann-Daszczuk B, Nguyen KCQ, Hall DH. 2008. Cell invasion and matricide during *Photorhabdus luminescens* transmission by *Heterorhabditis bacteriophora* nematodes. Appl Environ Microbiol 74:2275–2287. https://doi.org/10.1128/AEM.02646-07.
- 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.
- Lacey LA, Georgis R. 2012. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. J Nematol 44:218–225.
- Cox MP, Peterson DA, Biggs PJ. 2010. SolexaQA: at-a-glance quality assessment of Illumina second-generation sequencing data. BMC Bioinformatics 11:485. https://doi.org/10.1186/1471-2105-11-485.
- 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.
- 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 AL, 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.

- Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108. https://doi.org/10.1093/nar/ gkm160.
- 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.
- Brugirard-Ricaud K, Duchaud E, Givaudan A, Girard PA, Kunst F, Boemare N, Brehélin M, Zumbihl R. 2005. Site-specific antiphagocytic function of the *Photorhabdus luminescens* type III secretion system during insect colonization. Cell Microbiol 7:363–371. https://doi.org/10.1111/j.1462 -5822.2004.00466.x.
- Coombes BK. 2009. Type III secretion system in symbiotic adaptation of pathogenis and non-pathogenic bacteria. Trends Microbiol 7:89–94.
- Bowen DJ, Rocheleau TA, Grutzmacher CK, Meslet L, Valens M, Marble D, Dowling A, Ffrench-Constant R, Blight MA. 2003. Genetic and biochemical characterization of PrtA, and RTX-like metalloprotease from *Photorhabdus*. Microbiology 149:1581–1591. https://doi.org/10.1099/mic.0 .26171-0.
- Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/ AAC.02412-14.
- Watson RJ, Millichap P, Joyce SA, Reynolds S, Clarke DJ. 2010. The role of iron uptake in pathogenicity and symbiosis in *Photorhabdus luminescens* TT01. BMC Microbiol 10:177. https://doi.org/10.1186/1471-2180-10-177.