

Draft Genome Sequence of the Moderately Halophilic Bacterium Marinobacter lipolyticus Strain SM19

R. Thane Papke,^a Rafael R. de la Haba,^b Carmen Infante-Domínguez,^b Dolores Pérez,^b Cristina Sánchez-Porro,^b Pascal Lapierre,^c Antonio Ventosa^b

Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut, USA^a; Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, Spain^b; Center for Medical Science, New York Department of Health, Albany, New York, USA^c

Marinobacter lipolyticus strain SM19, isolated from saline soil in Spain, is a moderately halophilic bacterium belonging to the class *Gammaproteobacteria*. Here, we report the draft genome sequence of this strain, which consists of a 4.0-Mb chromosome and which is able to produce the halophilic enzyme lipase LipBL.

Received 7 May 2013 Accepted 21 May 2013 Published 27 June 2013

Citation Papke RT, de la Haba RR, Infante-Domínguez C, Pérez D, Sánchez-Porro C, Lapierre P, Ventosa A. 2013. Draft genome sequence of the moderately halophilic bacterium Marinobacter lipolyticus strain SM19. Genome Announc. 1(4):e00379-13. doi:10.1128/genomeA.00379-13.

Copyright © 2013 Papke et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Antonio Ventosa, ventosa@us.es.

"he genus Marinobacter, within the class Gammaproteobacteria, family Alteromonadaceae, currently includes 33 species that are isolated mainly from saline or hypersaline environments (marine habitats, salterns, etc.) (1, 2). Marinobacter lipolyticus is a moderately halophilic bacterium (3) isolated from saline soil in Cádiz, Spain (4), following a screening program that focused on isolating and characterizing halophilic bacteria that are capable of producing hydrolytic enzymes useful for biotechnological applications (5–9). M. lipolyticus grows optimally at 7.5% NaCl (range, 1 to 15%), 37°C (range, 15 to 40°C), and pH 7.5 (range, pH 5 to 10). It is able to produce a halophilic lipase, designated LipBL, which was expressed in Escherichia coli (3). Characterization of LipBL has demonstrated it to consist of 404 amino acids, with a molecular mass of 45.3 kDa, and to have high sequence identity with class C β-lactamases. Though M. lipolyticus is not a thermophile, the maximal temperature activity of LipBL was found to be 80°C, pH 7.0, and without NaCl (it maintained 20% activity in a wide range of NaCl concentrations). The highest activity observed was against short-to-medium-length acyl chain substrates, although it also hydrolyzed olive oil and fish oil (10). LipBL is an interesting enzyme, with potential biotechnological applications due to its high regioselectivity (10, 11). The role of conserved amino acids in the lipolytic activity of this family VIII lipase enzyme has been recently studied (12).

A draft genome sequence of M. lipolyticus strain SM19 was obtained using a whole-genome shotgun strategy (13) with an Illumina sequencing system (Illumina, Inc., San Diego, CA), which produced paired-end reads of ~45 bp, with an insert size of 300 bp. The final genome assembly has ~100-fold coverage of the entire genome. All reads were assembled into 35 supercontigs composed of 52 contigs (\geq 89 bp) using Velvet v1.0 (14). Forty-three (83%) of the contigs were \geq 510 bp and \leq 683,264 bp (average size, 93,563 bp) and were used to identify open reading frames (ORFs) and provide a functional annotation of predicted proteins, rRNA genes, and tRNA genes. Analysis was automated with Integrative Services for Genomic Analysis (ISGA) (15).

The genome is estimated to contain 4,023,208 bp, with a G+C content of 56.7%, and 3,646 putative ORFs with an average size of 995 bp. The assessed coding density is 90.1%. Furthermore, SM19 contains a single rRNA operon and a total of 46 tRNA genes.

The genome analysis confirms the presence of genes for lipase, amylase, protease, and DNase. Ectoine synthase and bacterial type I/II/III/IV/VI secretion systems were also present.

Nucleotide sequence accession number. The *M. lipolyticus* strain SM19 Whole-Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. ASAD000000000. The version described in this paper is the first version.

ACKNOWLEDGMENTS

This work was supported by grants from the National Science Foundation (award no. 0919290 and 080024), the Spanish Ministry of Science and Innovation (CGL2010-19303), and the Junta de Andalucía (P10-CVI-6226). FEDER funds also supported this project.

REFERENCES

- 1. Gauthier MJ, Lafay B, Christen R, Fernandez L, Acquaviva M, Bonin P, Bertrand JC. 1992. *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. Int. J. Syst. Bacteriol. 42:568–576.
- Bowman JP, McMeekin TA. 2005. Genus VII. Marinobacter, p 459–463. In Brenner DJ, Krieg NR, Staley JT, Garrity GM (ed), Bergey's manual of systematic bacteriology, 2nd ed, vol. 2, part B: the Gammaproteobacteria. Springer Verlag, New York, NY.
- Martín S, Márquez MC, Sánchez-Porro C, Mellado E, Arahal DR, Ventosa A. 2003. Marinobacter lipolyticus sp. nov., a novel moderate halophile with lipolytic activity. Int. J. Syst. Evol. Microbiol. 53:1383–1387.
- Ventosa A, Nieto JJ, Oren A. 1998. Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev. 62:504–544.
- Sánchez-Porro C, Martín S, Mellado E, Ventosa A. 2003. Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes. J. Appl. Microbiol. 94:295–300.
- Sánchez-Porro C, Mellado E, Bertoldo C, Antranikian G, Ventosa A. 2003. Screening and characterization of the protease CP1 produced by the moderately halophilic bacterium *Pseudoalteromonas* sp. strain CP76. Extremophiles 7:221–228.

- 7. Ventosa A, Sánchez-Porro C, Martín S, Mellado E. 2005. Halophilic archaea and bacteria as a source of extracellular hydrolytic enzymes, p 337–354. *In* Gunde-Cimerman N, Oren A, Plemenitas A (ed), Adaptation to life at high salt concentrations in Archaea, Bacteria, and Eukarya. Springer Verlag, Heidelberg, Berlin, Germany.
- 8. Mellado E, Martín S, Sánchez-Porro C, Ventosa A. 2005. Lipolytic enzymes from extremophilic microorganisms, p 25–43. *In* Mellado E, Barredo J-L (ed), Microorganisms for industrial enzymes and biocontrol. Research Signpost, Kerala, India.
- 9. Moreno ML, García MT, Ventosa A, Mellado E. 2009. Characterization of *Salicola* sp. IC10, a lipase- and protease-producing extreme halophile. FEMS Microbiol. Ecol. 68:59–71.
- Pérez D, Martín S, Fernández-Lorente G, Filice M, Guisán JM, Ventosa A, García MT, Mellado E. 2011. A novel halophilic lipase, LipBL, showing high efficiency in the production of eicosapentaenoic acid (EPA). PLoS One 6:e23325. doi:10.1371/journal.pone.0023325.
- Pérez D, Ventosa A, Mellado E, Guisán JM, Fernández-Lorente G, Filice M. 2010. Lipasa LipBL y sus aplicaciones. Spanish patent P201031636.

- Pérez D, Kovačić F, Wilhelm S, Jaeger KE, García MT, Ventosa A, Mellado E. 2012. Identification of amino acids involved in the hydrolytic activity of lipase LipBL from *Marinobacter lipolyticus*. Microbiology 158: 2192–2203.
- 13. Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, Bult CJ, Tomb JF, Dougherty BA, Merrick JM, McKenney K, Sutton G, FitzHugh W, Fields C, Gocyne JD, Scott J, Shirley R, Liu L-I, Glodek A, Kelley JM, Weidman JF, Phillips CA, Spriggs T, Hedblom E, Cotton MD, Utterback TR, Hanna MC, Nguyen DT, Saudek DM, Brandon RC, Fine LD, Fritchman JL, Fuhrmann JL, Geoghagen NSM, Gnehm CL, McDonald LA, Small KV, Fraser CM, Smith HO, Venter JC. 1995. Whole-genome random sequencing and assembly of Haemophilus influenza Rd. Science 269:496–512.
- 14. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res. 18:821–829.
- Hemmerich C, Buechlein A, Podicheti R, Revanna KV, Dong Q. 2010.
 An Ergatis-based prokaryotic genome annotation web server. Bioinformatics 26:1122–1124.