

## Genome Sequence of the Cheese-Starter Strain Lactobacillus delbrueckii subsp. lactis CRL 581

Elvira María Hebert, Raúl R. Raya, Lucía Brown, Graciela Font de Valdez, Graciela Savoy de Giori, María Pía Taranto

Centro de Referencia para Lactobacilos (CERELA-CONICET), San Miguel de Tucumán, Argentina

We report the genome sequence of *Lactobacillus delbrueckii* subsp. *lactis* CRL 581 (1,911,137 bp, GC 49.7%), a proteolytic strain isolated from a homemade Argentinian hard cheese which has a key role in bacterial nutrition and releases bioactive health-beneficial peptides from milk proteins.

Received 4 July 2013 Accepted 11 July 2013 Published 8 August 2013

Citation Hebert EM, Raya RR, Brown L, Font de Valdez G, Savoy de Giori G, Taranto MP. 2013. Genome sequence of the cheese-starter strain *Lactobacillus delbrueckii* subsp. *lactis* CRL 581. Genome Announc. 1(4):e00602-13. doi:10.1128/genomeA.00602-13.

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

Address correspondence to Elvira María Hebert, ehebert@cerela.org.ar.

Lactobacillus delbrueckii subsp. lactis and L. delbrueckii subsp. bulgaricus are homofermentative thermophilic lactic acid bacteria (LAB) widely used as starter cultures for the manufacture of a variety of fermented dairy products, such as fermented sour milks and Swiss and Italian type cheeses (1). To date, the genome analyses of L. delbrueckii subsp. bulgaricus strains ATCC 11842, 2038, NDO2, and ATCC BAA-365 have been published (2–6). However, the genome analysis of L. delbrueckii subsp. lactis has not been published.

L. delbrueckii subsp. lactis strain CRL 581, isolated from a homemade Argentinian hard cheese (1), possesses an efficient proteolytic system which has a key role in bacterial nutrition as well as contributing to the development of the organoleptic properties of fermented milk products and releasing bioactive health-beneficial peptides (i.e., anti-inflammatory, antihypertensive, and phosphopeptides) from milk proteins (7, 8). The proteolytic system of the CRL 581 strain provides opportunities for the development of novel functional food with potential health-promoting properties. Here, we describe the genome sequence of L. delbrueckii subsp. lactis CRL 581.

Whole-genome sequencing of L. delbrueckii subsp. lactis CRL 581 was performed with a 454 GS Titanium pyrosequencer at INDEAR, Argentina. Genomic libraries containing 8-kb inserts were prepared, and 122,806 paired-end reads and 221,348 singleend reads were generated using the 454 GS system, giving 42-fold coverage of the genome. Approximately 99.2% of these reads were assembled into 16 large scaffolds, including 163 nonredundant contigs, using version 2.6 of the 454 Newbler assembler (454 Life Sciences, Branford, CT). The draft genome is a single circular chromosome of 1,911,137 bases in length, with a mean GC content of 49.7%; no plasmids were observed in this genome. Genome annotation was performed by use of the standard operating procedures (SOPs) for prokaryotic annotation from ISGA (9), the RAST annotation server (10), the Glimmer 3.02 modeling software package (11), tRNAscan-SE 1.21 (12), and RNAmmer 1.2 (13). A total of 1,880 coding sequences (CDS), 37 structural tRNAs, and 3 rRNA operons were predicted. There are 254 RAST subsystems represented in the chromosome. Additionally, no

functional prophages were identified, although several genes for transposases and two clusters of regularly interspaced short palindromic repeats (CRISPRs) and two potential CRISPRs were found.

The genome of *L. delbrueckii* subsp. *lactis* CRL 581 encodes several components of the proteolytic system, including the cell envelope-associated proteinase (PrtL), aminopeptidases (PepC, PepN, PepM, and PepA), endopeptidases (PepO and PepF), dipeptidases (PepD and PepV), tripeptidases (PepT), and proline peptidases (PepX, PepI, PepP and PepQ) as well as the di/tripeptide Dpp and the oligopeptide Opp systems. The presence of these enzymes supports the role of the cheese starter culture *L. delbrueckii* subsp. *lactis* CRL 581 in milk.

Comparative genome analysis of *L. delbrueckii* subsp. *lactis* CRL 581 with the closely related *L. delbrueckii* subsp. *bulgaricus* strains revealed that they are highly similar. However, proteins and enzymes involved in galactose, sucrose, maltose, and trehalose utilization are present only in the *L. delbrueckii* subsp. *lactis* CRL 581 genome.

This is the first report of the genome sequence of a technologically relevant *L. delbrueckii* subsp. *lactis* strain; these data will be useful for exploration of its biotechnological properties.

**Nucleotide sequence accession numbers.** The data from this whole-genome shotgun project were deposited in GenBank under the accession number ATBQ000000000. The version described in this paper is version ATBQ00000000.1.

## **ACKNOWLEDGMENTS**

This work was supported by grants from CONICET, FONARSEC, and ANPCyT-MINCyT, Argentina.

## **REFERENCES**

- 1. Hébert EM, Raya RR, Tailliez P, de Giori GS. 2000. Characterization of natural isolates of *Lactobacillus* strains to be used as starter cultures in dairy fermentation. Int. J. Food Microbiol. 59:19–27.
- Hao P, Zheng H, Yu Y, Ding G, Gu W, Chen S, Yu Z, Ren S, Oda M, Konno T, Wang S, Li X, Ji ZS, Zhao G. 2011. Complete sequencing and pan-genomic analysis of *Lactobacillus delbrueckii* subsp. *bulgaricus* reveal its genetic basis for industrial yogurt production. PLoS One 6:e15964. doi: 10.1371/journal.pone.0015964.

- 3. van de Guchte M, Penaud S, Grimaldi C, Barbe V, Bryson K, Nicolas P, Robert C, Oztas S, Mangenot S, Couloux A, Loux V, Dervyn R, Bossy R, Bolotin A, Batto JM, Walunas T, Gibrat JF, Bessières P, Weissenbach J, Ehrlich SD, Maguin E. 2006. The complete genome sequence of *Lactobacillus bulgaricus* reveals extensive and ongoing reductive evolution. Proc. Natl. Acad. Sci. U. S. A. 103:9274–9279.
- Sun Z, Chen X, Wang J, Zhao W, Shao Y, Guo Z, Zhang X, Zhou Z, Sun T, Wang L, Meng H, Zhang H, Chen W. 2011. Complete genome sequence of *Lactobacillus delbrueckii* subsp. *bulgaricus* strain ND02. J. Bacteriol. 193:3426–3427.
- 5. Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E, Pavlov A, Pavlova N, Karamychev V, Polouchine N, Shakhova V, Grigoriev I, Lou Y, Rohksar D, Lucas S, Huang K, Goodstein DM, Hawkins T, Plengvidhya V, Welker D, Hughes J, Goh Y, Benson A, Baldwin K, Lee JH, Díaz-Muñiz I, Dosti B, Smeianov V, Wechter W, Barabote R, Lorca G, Altermann E, Barrangou R, Ganesan B, Xie Y, Rawsthorne H, Tamir D, Parker C, Breidt F, Broadbent J, Hutkins R, O'Sullivan D, Steele J, Unlu G, Saier M, Klaenhammer T, Richardson P, Kozyavkin S, Weimer B, Mills D. 2006. Comparative genomics of the lactic acid bacteria. Proc. Natl. Acad. Sci. U. S. A. 103:15611–15616.
- 6. McNulty NP, Yatsunenko T, Hsiao A, Faith JJ, Muegge BD, Goodman AL, Henrissat B, Oozeer R, Cools-Portier S, Gobert G, Chervaux C, Knights D, Lozupone CA, Knight R, Duncan AE, Bain JR, Muehlbauer MJ, Newgard CB, Heath AC, Gordon JI. 2011. The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. Sci. Transl. Med. 3:106ra106. doi:10.1126/scitranslmed.3002701.
- 7. Hebert EM, Mamone G, Picariello G, Raya RR, Savoy G, Ferranti P,

- Addeo F. 2008. Characterization of the pattern of  $\alpha_{s1}$  and  $\beta$ -casein breakdown and release of a bioactive peptide by a cell envelope proteinase from *Lactobacillus delbrueckii* subsp. *lactis* CRL 581. Appl. Environ. Microbiol. 74:3682–3689.
- Espeche Turbay MB, de Moreno de LeBlanc A, Perdigón G, Savoy de Giori G, Hebert EM. 2012. β-Casein hydrolysate generated by the cell envelope-associated proteinase of *Lactobacillus delbrueckii* ssp. *lactis* CRL 581 protects against trinitrobenzene sulfonic acid-induced colitis in mice. J. Dairy Sci. 95:1108–1118.
- Hemmerich C, Buechlein A, Podicheti R, Revanna KV, Dong Q. 2010.
  An Ergatis-based prokaryotic genome annotation web server. Bioinformatics 26:1122–1124.
- 10. 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.
- 11. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25: 955–964.
- 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.