

Draft Genome Sequence of *Lactobacillus delbrueckii* subsp. *bulgaricus* CFL1, a Lactic Acid Bacterium Isolated from French Handcrafted Fermented Milk

Julie Meneghel,^a  Eric Dugat-Bony,^a Françoise Irlinger,^a Valentin Loux,^b Marie Vidal,^{c,d} Stéphanie Passot,^a Catherine Béal,^a Séverine Layec,^a Fernanda Fonseca^a

UMR GMPA, AgroParisTech, INRA, Université Paris-Saclay, Thiverval-Grignon, France^a; MaIAGE, INRA, Université Paris-Saclay, Jouy-en-Josas, France^b; INRA, GeT-PlaGe, Genotoul, Castanet-Tolosan, France^c; INRA, UAR1209, Castanet-Tolosan, France^d

Lactobacillus delbrueckii subsp. *bulgaricus* (*L. bulgaricus*) is a lactic acid bacterium widely used for the production of yogurt and cheeses. Here, we report the genome sequence of *L. bulgaricus* CFL1 to improve our knowledge on its stress-induced damages following production and end-use processes.

Received 15 January 2016 Accepted 20 January 2016 Published 3 March 2016

Citation Meneghel J, Dugat-Bony E, Irlinger F, Loux V, Vidal M, Passot S, Béal C, Layec S, Fonseca F. 2016. Draft genome sequence of *Lactobacillus delbrueckii* subsp. *bulgaricus* CFL1, a lactic acid bacterium isolated from French handcrafted fermented milk. *Genome Announc* 4(2):e00052-16. doi:10.1128/genomeA.00052-16.

Copyright © 2016 Meneghel 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 Fernanda Fonseca, fonseca@grignon.inra.fr.

Lactobacillus delbrueckii subsp. *bulgaricus* (*L. bulgaricus*) is a lactic acid bacterium that is traditionally used as a dairy starter. *L. bulgaricus* is mainly involved in milk acidification and more generally plays a role in the preservation and development of flavor, texture, and vitamins of dairy products (1, 2). In addition, this bacterium is exploited to produce lactic acid for use in the food, cosmetics, and chemical sectors (3).

Aimed at enhancing its industrial utilization, considerable research efforts have been undertaken to characterize the diversity of *L. bulgaricus* strains. Since 2006, the genome sequences of six different strains were published: ATCC 11842 (1), ATCC BAA-365 (4), 2038 (5), CNCM I-1519 and CNCM I-1632 (6), and CRL871 (7). Actually, the production of lactic acid bacteria by traditional fermentation, their stabilization (by freezing and/or drying methods), and final use (in yogurt, cheese-making, or biomolecule production) are responsible for the undesirable degradation of their viability and functionality. Therefore, scientific work has also focused on optimizing the formulation and operating conditions of starter production and the study of *L. bulgaricus* behavior under related stress conditions, especially considering the sensitive strain CFL1 (8–16). However, the majority of these studies are descriptive, and there is a clear need for comprehension of the underlying molecular cellular damage mechanisms.

Consequently, we sequenced the genome of *L. bulgaricus* CFL1 (available at CIRM-BIA, Rennes, France), isolated >20 years ago from handcrafted fermented milk, by using Illumina MiSeq technology. Paired-end sequences (2 × 250-bp long) were merged using FLASH (17) and assembled using SPAdes (version 3.1.1, with default parameters) (18), which generated 44 large contigs (>1,000 bp), with an average sequencing coverage of 115-fold, N_{50} contig of 116 Kbp, and a largest contig of 266 Kbp. The unclosed draft genome is 1,757,917 bp in length and has a G+C content of 49.8%. Gene prediction and annotation were performed using the Inte-

grated Microbial Genomes (IMG) system (19), according to the standard operating procedure of the Department of Energy-Joint Genome Institute (DOE-JGI) microbial genome annotation pipeline (MGAP version 4) (20). The genome is composed of 1,882 predicted genes comprising 1,794 coding DNA sequences, 20 rRNAs, and 68 tRNAs.

The analysis of the genome of *L. bulgaricus* CFL1 revealed general consistency with previously sequenced strains, including G+C content, carbohydrate metabolism, and genes involved in exopolysaccharide and lipopolysaccharide production. However, a disparity regarding the detected clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) systems should be emphasized. They consist of defense systems against plasmid and phage invasions that are classified into three major groups (types I, II, and III) (21). Whereas in *L. bulgaricus*, only type II or III seems to be present, if any (22), interestingly, CFL1 appeared to possess both of these CRISPR-Cas system types simultaneously.

The availability of the genome sequence and annotation of *L. bulgaricus* CFL1 will allow a deeper insight into its stress response mechanisms, thus making it possible to identify potential preservation issues and to better understand cell damages following production and end-use processes, especially through transcriptomic and proteomic approaches.

Nucleotide sequence accession numbers. The draft genome sequences of *L. bulgaricus* CFL1 have been deposited at the EMBL database under accession numbers CZPS01000001 to CZPS01000044.

ACKNOWLEDGMENTS

We thank the INRA GeT-PlaGE platform (<http://get.genotoul.fr>) for sequencing the genome, the INRA MIGALE bioinformatics platform (<http://migale.jouy.inra.fr>) for providing computational resources and support, and the IMG E/R pipeline for generating annotations.

REFERENCES

- van de Guchte M, Penaud S, Grimaldi C, Barbe V, Bryson K, Nicolas P, Robert C, Oztas S, Mangelot S, Couloux A, Loux V, Dervyn R, Bossy R, Bolotin A, Batto J-M, Walunas T, Gibrat J-F, Bessi res 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 USA* 103:9274–9279. <http://dx.doi.org/10.1073/pnas.0603024103>.
- Ott A, Germond J-E, Chaintreau A. 2000. Origin of acetaldehyde during milk fermentation using ¹³C-labeled precursors. *J Agric Food Chem* 48: 1512–1517. <http://dx.doi.org/10.1021/jf9904867>.
- Datta R, Henry M. 2006. Lactic acid: recent advances in products, processes and technologies—a review. *J Chem Technol Biotechnol* 81: 1119–1129. <http://dx.doi.org/10.1002/jctb.1486>.
- 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 J-H, Diaz-Muniz 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 USA* 103:15611–15616. <http://dx.doi.org/10.1073/pnas.0607117103>.
- 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 Z-S, 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. <http://dx.doi.org/10.1371/journal.pone.0015964>.
- McNulty NP, Yatsunenkov 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. <http://dx.doi.org/10.1126/scitranslmed.3002701>.
- Lai o JE, Hebert EM, de Giori GS, LeBlanc JG. 2015. Draft genome sequence of *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL871, a folate-producing strain isolated from a northwestern Argentinian yogurt. *Genome Announc* 3(3):e00693–15. <http://dx.doi.org/10.1128/genomeA.00693-15>.
- Fonseca F, B  l C, Corrieu G. 2000. Method of quantifying the loss of acidification activity of lactic acid starters during freezing and frozen storage. *J Dairy Res* 67:83–90. <http://dx.doi.org/10.1017/S002202999900401X>.
- Oldenhof H, Wolters WF, Fonseca F, Passot S, Marin M. 2005. Effect of sucrose and maltodextrin on the physical properties and survival of air-dried *Lactobacillus bulgaricus*: an *in situ* Fourier transform infrared spectroscopy study. *Biotechnol Prog* 21:885–892. <http://dx.doi.org/10.1021/bp049559j>.
- Fonseca F, Marin M, Morris GJ. 2006. Stabilization of frozen *Lactobacillus delbrueckii* subsp. *bulgaricus* in glycerol suspensions: freezing kinetics and storage temperature effects. *Appl Environ Microbiol* 72:6474–6482. <http://dx.doi.org/10.1128/AEM.00998-06>.
- Streit F, Delettre J, Corrieu G, B  l C. 2008. Acid adaptation of *Lactobacillus delbrueckii* subsp. *bulgaricus* induces physiological responses at membrane and cytosolic levels that improves cryotolerance. *J Appl Microbiol* 105:1071–1080. <http://dx.doi.org/10.1111/j.1365-2672.2008.03848.x>.
- Rault A, Bouix M, B  l C. 2009. Fermentation pH influences the physiological-state dynamics of *Lactobacillus bulgaricus* CFL1 during pH-controlled culture. *Appl Environ Microbiol* 75:4374–4381. <http://dx.doi.org/10.1128/AEM.02725-08>.
- Passot S, Cenard S, Douania I, Tr    l IC, Fonseca F. 2012. Critical water activity and amorphous state for optimal preservation of lyophilised lactic acid bacteria. *Food Chem* 132:1699–1705. <http://dx.doi.org/10.1016/j.foodchem.2011.06.012>.
- Gautier J, Passot S, P  nicaud C, Guillemin H, Cenard S, Lieben P, Fonseca F. 2013. A low membrane lipid phase transition temperature is associated with a high cryotolerance of *Lactobacillus delbrueckii* subspecies *bulgaricus* CFL1. *J Dairy Sci* 96:5591–5602. <http://dx.doi.org/10.3168/jds.2013-6802>.
- Passot S, Jamme F, R  f  regiers M, Gautier J, Cenard S, Fonseca F. 2014. Synchrotron UV fluorescence microscopy for determining membrane fluidity modification of single bacteria with temperatures. *Biomed Spectrosc Imaging* 3:203–210.
- Passot S, Gautier J, Jamme F, Cenard S, Dumas P, Fonseca F. 2015. Understanding the cryotolerance of lactic acid bacteria using combined synchrotron infrared and fluorescence microscopies. *Analyst* 140: 5920–5928. <http://dx.doi.org/10.1039/c5an00654f>.
- Mago   T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27:2957–2963. <http://dx.doi.org/10.1093/bioinformatics/btr507>.
- Bankovich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski 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. <http://dx.doi.org/10.1089/cmb.2012.0021>.
- Markowitz VM, Chen I-MA, Palaniappan K, Chu K, Szeto E, Grechkin Y, Ratner A, Jacob B, Huang J, Williams P, Huntemann M, Anderson I, Mavromatis K, Ivanova NN, Kyrpides NC. 2012. IMG: the integrated microbial genomes database and comparative analysis system. *Nucleic Acids Res* 40:D115–D122. <http://dx.doi.org/10.1093/nar/gkr1044>.
- Mavromatis K, Ivanova NN, Chen IM, Szeto E, Markowitz VM, Kyrpides NC. 2009. The DOE-JGI standard operating procedure for the annotations of microbial genomes. *Stand Genomic Sci* 1:63–67. <http://dx.doi.org/10.4056/sigs.632>.
- Bhaya D, Davison M, Barrangou R. 2011. CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. *Annu Rev Genet* 45:273–297. <http://dx.doi.org/10.1146/annurev-genet-110410-132430>.
- Urshev Z, Ishlimova D. 2015. Distribution of clustered regularly interspaced palindrome repeats CRISPR2 and CRISPR3 in *Lactobacillus delbrueckii* ssp. *bulgaricus* strains. *Biotechnol Biotechnol Equip* 29:541–546. <http://dx.doi.org/10.1080/13102818.2015.1013351>.