

# Genome Sequence of *Lactobacillus brevis* Strain D6, Isolated from Smoked Fresh Cheese

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**The autochthonous *Lactobacillus brevis* strain D6, isolated from smoked fresh cheese, carries a 45-kDa S-layer protein. Strain D6 has shown adhesion to extracellular matrix proteins and to Caco-2 intestinal epithelial cells, as well as immunomodulatory potential and beneficial milk technological properties. Hence, it could be used as a potential probiotic starter culture for cheese production.**

Received 23 February 2016 Accepted 24 February 2016 Published 7 April 2016

**Citation** Kant R, Uroić K, Hynönen U, Kos B, Šušković J, Palva A. 2016. Genome sequence of *Lactobacillus brevis* strain D6, isolated from smoked fresh cheese. *Genome Announc* 4(2):e00264-16. doi:10.1128/genomeA.00264-16.

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An autochthonous *Lactobacillus brevis* strain, D6, was isolated from traditionally produced smoked fresh cheese originating from a specific Croatian ecological locality of the Zagorje region. These artisanal cheeses are produced by the spontaneous fermentation of cow's milk and harbor a rich diversity of lactic acid bacteria (1). As they are manufactured from non-pasteurized milk without using commercial starter cultures, their sensory properties depend on the composition of lactic acid bacteria in raw milk, where the present bacteria act as natural starter cultures (2). *Lactobacillus brevis* is an abundant *Lactobacillus* species in traditionally produced smoked fresh cheeses. It is a microaerophilic, Gram-positive and obligatory hetero-fermentative lactic acid bacterium. It has been isolated from many different environments such as sauerkraut, sourdough, silage, and the intestinal tract of humans and animals, and it is involved in the production of a wide spectrum of fermented products (3, 4). For instance, as a hetero-fermentative bacterium, *L. brevis* plays a major role in the fermentation of Italian sweet doughs during the production of Panettone, Colomba, and Pandoro breads and various mini-cakes, where it is a predominant starter culture in the mixture of lactic acid bacteria used (5).

Some *L. brevis* strains with specific surface (S-layer) proteins possess a variety of functional properties that make them both potential probiotics and good vaccine vector candidates (6, 7). The strain *L. brevis* D6 carries a 45-kDa S-layer protein and has shown adhesion to extracellular matrix proteins and to Caco-2 intestinal epithelial cells, as well as immunomodulatory potential. Together with the putative protective role of S-layer proteins, suggested by the enhanced survival of S-layer carrying *L. brevis* D6 in rigorous conditions, such as those encountered in the gastrointestinal tract and during the process of freeze-drying, this strain could be defined as a potential probiotic starter culture for cheese production (8).

The genomic DNA of *L. brevis* D6 was extracted from 1 mL of overnight culture grown in MRS medium using the Wizard R Genomic DNA purification kit (Promega) and the entire genome was sequenced using the Roche 454 Life Sciences GS FLX system. The obtained sequences were assembled using Newbler, ending

up with a 12× coverage of each genome. Annotation was performed based on the PGAAP analysis ([http://www.ncbi.nlm.nih.gov/genomes/static/Annotation\\_pipeline\\_procedures.txt](http://www.ncbi.nlm.nih.gov/genomes/static/Annotation_pipeline_procedures.txt)) and manual verification.

The draft genome of *L. brevis* D6 strain contains 2,591,675 nucleotides with an overall G+C content of 45.6% in 123 contigs. The chromosome contains 2,569 protein-coding sequences (CDS) with 76 RNA genes, 121 pseudo genes, and one clustered regularly interspaced short palindromic repeat (CRISPR) array. Using the SignalP software (9), 140 proteins in total were predicted to be secreted.

**Nucleotide sequence accession number.** The draft genome sequence of *Lactobacillus brevis* strain D6 is available in GenBank under the accession number [LQNG00000000](https://www.ncbi.nlm.nih.gov/nuclseq/LQNG00000000).

## ACKNOWLEDGMENTS

We thank the Sequencing Laboratory, Institute of Biotechnology, University of Helsinki, Finland for the sequencing.

We thank the Ministry of Science, Education and Sports of the Republic of Croatia (project No. 058-0581990-2007) and Croatian Science Foundation (project No. IP-2014-09-7009) for financial support. This work was further supported by the Academy of Finland (AF)-funded Center of Excellence in Microbial Food Safety (CoE-MiFoSa) research program (grants 118602 and 141140), and by general AF research grant (277362) (<http://www.aka.fi>).

## FUNDING INFORMATION

This work, including the efforts of Ravi Kant, Ulla Hynönen, and Airi Palva, was funded by Academy of Finland (118602 and 141140). This work, including the efforts of Ksenija Uroić and Blaženka Kos, was funded by Ministry of Science, Education and Sports of Republic of Croatia (058-0581990-2007). This work, including the efforts of Ksenija Uroić, Blaženka Kos, and Jagoda Šušković, was funded by Croatian Science Foundation (IP-2014-09-7009).

## REFERENCES

1. Uroić K. 2014. The role of S-proteins in probiotic lactic acid bacteria. Ph.D. thesis. Faculty of Food Technology and Biotechnology, University of Zagreb.

2. Leboš Pavunc A, Beganović J, Kos B, Uroić K, Blažić M, Šušćković J. 2012. Characterization and application of autochthonous starter cultures for fresh cheese production. *Food Technol Biotechnol* 50:141–151.
3. Uroić K, Nikolić M, Kos B, Leboš Pavunc A, Beganović J, Lukić J, Jovčić B, Filipić B, Miljković M, Golić N, Topisirović Lj, Čadež N, Raspor P, Šušćković J. 2014. Probiotic properties of lactic acid bacteria isolated from Croatian fresh soft cheeses and Serbian white-pickled cheeses. *Food Technol Biotechnol* 52:232–241.
4. Beganović J, Kos B, Leboš Pavunc A, Uroić K, Jokić M, Šušćković J. 2014. Traditionally produced sauerkraut as source of autochthonous functional starter cultures. *Microbiol Res* 169:623–632. <http://dx.doi.org/10.1016/j.micres.2013.09.015>.
5. Vogel FV, Nyanga LK. 2005. Sourdough fermentation. In *Food fermentation*, Nout RMJ, de Vos WM, Zwietering MH (ed). Wageningen Academic Publishers, Wageningen, the Netherlands.
6. Läheteinen T, Lindholm A, Rinttilä T, Junnikkala S, Kant R, Pietilä TE, Levonen K, von Ossowski I, Solano-Aguilar G, Jakava-Viljanen M, Palva A. 2014. Effect of *Lactobacillus brevis* ATCC 8287 as a feeding supplement on the performance and immune function of piglets. *Vet Immunol Immunopathol* 158:14–25. <http://dx.doi.org/10.1016/j.vetimm.2013.09.002>.
7. Hynönen U, Kant R, Läheteinen T, Pietilä TE, Beganović J, Smidt H, Uroić K, Ävall-Jääskeläinen S, Palva A. 2014. Functional characterization of probiotic surface layer protein-carrying *Lactobacillus amylovorus* strains. *BMC Microbiol* 14:199. <http://dx.doi.org/10.1186/1471-2180-14-199>.
8. Uroić K, Beganović J, Hynönen U, Pietilä TE, Leboš Pavunc A, Kant R, Kos B, Palva A, Šušćković J. 2016. The role of S-layer in adhesive and immunological properties of probiotic starter culture *Lactobacillus brevis* D6 isolated from artisanal smoked fresh cheese. *LWT Food Sci Technol* 69:623–632.
9. Petersen TN, Brunak S, von Heijne G, Nielsen H. 2011. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat Methods* 8:785–786. <http://dx.doi.org/10.1038/nmeth.1701>.