

Complete Genome Sequencing of *Lactobacillus acidophilus* 30SC, Isolated from Swine Intestine[∇]

Sejong Oh,¹§ Hanseong Roh,²§ Hyeok-Jin Ko,² Sujin Kim,² Kyoung Heon Kim,²
Sung Eun Lee,³ In Seop Chang,⁴ Saehun Kim,² and In-Geol Choi^{2*}

Division of Animal Science, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju 500-757, South Korea¹; College of Life Sciences and Biotechnology, Korea University, Anam-dong, Seongbuk-gu, Seoul 136-713, South Korea²; Research Station, Nanototech Inc., Gyunggi-Technopark Technology Development Center, Ansan 426-901, South Korea³; and School of Environmental Science and Engineering, Gwangju Institute of Science and Technology, 261 Cheomdan-gwagi-ro, Buk-gu, Gwangju 500-712, South Korea⁴

Received 11 March 2011/Accepted 16 March 2011

***Lactobacillus acidophilus* 30SC has been isolated from swine intestines and considered a probiotic strain for dairy products because of its ability to assimilate cholesterol and produce bacteriocins. Here, we report the complete genome sequence of *Lactobacillus acidophilus* 30SC (2,078,001 bp) exhibiting strong acid resistance and enhanced bile tolerance.**

Lactobacillus acidophilus 30SC is an intestinal isolate from swine and known to assimilate cholesterol and produce a thermostable and pH-stable bacteriocin (9). This bacteriocin may have a potential application in food systems controlling food spoilage microorganisms and food-borne pathogens (3, 9). In addition, *L. acidophilus* 30SC showed excellent acid resistance and enhanced bile tolerance compared to commercial probiotic strains that are presently used in dairy products (9). These properties are advantageous characteristics to a probiotic strain by providing a greater chance of survival during passage through the stomach and intestinal tract. In order to understand these unique properties on the genomic level, we attempted whole-genome sequencing.

The genome of *L. acidophilus* 30SC was sequenced by Roche 454 GS FLX (454 GS FLX) platforms. The initial draft assembly was prepared from two separate libraries of 158,188 and 235,924 paired-end reads containing 3-kb inserts and yielded 15 scaffolds (21 contigs) by the Newbler gsAssembler 2.3 (454 Life Sciences, Branford, CT). The actual order of scaffolds was checked by a series of PCRs based on a permutation table. The genome sequence was finished by filling gaps with gapResolution (Cliff Han, unpublished data; provided by the Joint Genome Institute) and sequencing by ABI system (Applied Biosystems, CA) followed by primer walking PCR between contigs.

The complete genome sequence of *L. acidophilus* 30SC revealed one circular chromosome of 2,078,001 bp with a GC content of 38.1%. Two circular plasmids that have not been reported were also identified: pRKC30SC1 (7,197 bp) and pRKC30SC2 (12,568 bp), with GC content values of 35.1% and 36.6%, respectively. The full annotation was performed by a RAST server (1) and NCBI Prokaryotic Genomes Automatic

Annotation Pipeline (PGAAP) (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>). Both annotations were scrutinized with manual inspection. The chromosome has 2,138 coding sequences (CDS), 63 tRNA genes, and 12 rRNA genes. The pRKC30SC1 and pRKC30SC2 plasmids have 7 CDS and 16 CDS, respectively.

The genome sequencing revealed genes known to be implicated in bile acid tolerance, such as choloylglycine hydrolase, known as bile salt hydrolase and bile acid-inducible operon protein (4, 10). As in *Lactobacillus amylovorus*, which has recently been sequenced (7), we found two kinds of S-layer proteins, mucus- and fibronectin-binding proteins participating in adhesion to intestinal cells (2, 6) and five genes encoding bacteriocins, such as amylovorin (5) and helveticin (11), that can inhibit the growth of pathogenic bacteria (8), suggesting its potential as a probiotic bacterium.

Nucleotide sequence accession numbers. The complete genome sequence of *L. acidophilus* 30SC has been deposited in NCBI GenBank under accession numbers CP002559, CP002560, and CP002561.

This research was supported by the Korea Research Foundation (grant 314-2008-1-C00377), the Basic Science Research Program (2009-0068606), and the Global Frontier Project (Advanced Biomass) funded by the National Research Foundation (NRF) and the Korea Research Foundation grant to the South Korean government (MEST).

REFERENCES

1. Aziz, R., et al. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* **9**:75.
2. Buck, B. L., E. Altermann, T. Svingerud, and T. R. Klaenhammer. 2005. Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCFM. *Appl. Environ. Microbiol.* **71**:8344–8351.
3. De Vuyst, L., and F. Leroy. 2007. Bacteriocins from lactic acid bacteria: production, purification, and food applications. *J. Mol. Microbiol. Biotechnol.* **13**:194–199.
4. Elkins, C. A., and D. C. Savage. 1998. Identification of genes encoding conjugated bile salt hydrolase and transport in *Lactobacillus johnsonii* 100-100. *J. Bacteriol.* **180**:4344–4349.
5. Foulquié Moreno, M. R., B. Baert, S. Denayer, P. Cornelis, and L. De Vuyst. 2008. Characterization of the amylovorin locus of *Lactobacillus amylovorus* DCE 471, producer of a bacteriocin active against *Pseudomonas aeruginosa*, in combination with colistin and pyocins. *FEMS Microbiol. Lett.* **286**:199–206.

* Corresponding author. Mailing address: College of Life Sciences and Biotechnology, Korea University, Anam-dong, Seongbuk-gu, Seoul 136-713, South Korea. Phone and fax: 82 2 3290 3631. E-mail: igchoi@korea.ac.kr.

§ These two authors equally contributed to this work.

∇ Published ahead of print on 8 April 2011.

6. **Hynonen, U., B. Westerlund-Wikstrom, A. Palva, and T. K. Korhonen.** 2002. Identification by flagellum display of an epithelial cell- and fibronectin-binding function in the SlpA surface protein of *Lactobacillus brevis*. *J. Bacteriol.* **184**:3360–3367.
7. **Kant, R., L. Paulin, E. Alatalo, W. M. de Vos, and A. Palva.** 2011. Genome sequence of *Lactobacillus amylovorus* GRL1112. *J. Bacteriol.* **193**:789–790.
8. **Kim, Y., et al.** 2006. Inhibitory effects of *Lactobacillus acidophilus* lysates on the cytotoxic activity of Shiga-like toxin 2 produced from *Escherichia coli* O157:H7. *Lett. Appl. Microbiol.* **43**:502–507.
9. **Oh, S., S. H. Kim, and R. W. Worobo.** 2000. Characterization and purification of a bacteriocin produced by a potential probiotic culture, *Lactobacillus acidophilus* 30SC. *J. Dairy Sci.* **83**:2747–2752.
10. **Pfeiler, E. A., M. A. Azcarate-Peril, and T. R. Klaenhammer.** 2007. Characterization of a novel bile-inducible operon encoding a two-component regulatory system in *Lactobacillus acidophilus*. *J. Bacteriol.* **189**:4624–4634.
11. **Thompson, J. K., M. A. Collins, and W. D. Mercer.** 1996. Characterization of a proteinaceous antimicrobial produced by *Lactobacillus helveticus* CNRZ450. *J. Appl. Bacteriol.* **80**:338–348.