Complete genome sequence of *Lactobacillus amylovorus* 1394N20, a potential probiotic strain, isolated from a Hanwoo calf

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Abstract

Lactobacillus amylovorus are known to exist in the intestinal flora of healthy cattle or pigs. The *L. amylovorus* strain 1394N20 was isolated from the feces of the Hanwoo calf (*Bos taurus coreanae*). The genome of strain 1394N20 consists of a single circular chromosome (2,176,326 bp) with overall guanine + cytosine content of 37.8 mol%. Moreover, 2,281 protein-coding sequences, 15 rRNAs, and 65 tRNAs genes were identified in the chromosome based on the results of annotation. The bacterium has a gene encoding endoglucanase, an enzyme that hydrolyzes the 1,4-β-D-glycosidic linkages in cellulose, hemicellulose, lichenin, and cereal β-D-glucans. Genomic sequencing of *L. amylovorus* strain 1394N20 reveals the immense potential of the strain as a probiotic with nutrient digestibility.

Keywords: *Lactobacillus amylovorus*, Hanwoo calf, Whole genome sequencing, Endoglucanase

According to a recent study, microbial colonization of the intestine by diverse microbiota begins before birth in mammals; however, the microbiota changes rapidly in the early postnatal life [1]. *Lactobacillus*, a microorganism found in the intestinal flora of mammals such as cows and pigs, has the ability to inhibit the growth of pathogenic microorganisms by lowering the colon pH, and hence is widely used as probiotics [2]. Several microorganisms use cellulose as a carbon source, which is a major component of plants. Cellulose can be hydrolyzed by cellulase, which is composed of β-1,4-glycosidic bonds, promoting the composting of organic matter and improving feed efficiency by increasing its bioavailability in the intestine of livestock [3]. The most important source of energy in ruminant diets are carbohydrates, which are major precursors of lactose and fat in milk. Microorganisms present in the rumen facilitate the use of energy from fibrinous carbohydrates, such as cellulose and hemicellulose, which are bound to fibrin and lignins present in the cell walls of plants [4].

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Oh YJ, Kim JY, Lee J, Choi HJ.
Data curation: Oh YJ, Kim JY.
Formal analysis: Oh YJ, Lee J.
Methodology: Oh YJ, Lim SK.
Validation: Park J, Choi HJ.
Investigation Oh YJ, Lee J.
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Writing - review & editing: Park J, Choi HJ.

Ethics approval and consent to participate
This article does not require IRB/IACUC approval because there are no human and animal participants.

In this study, *Lactobacillus amylovorus* strain 1394N20 (KCCM 12999P) was isolated from the feces of a 8-day-old healthy male Hanwoo calf. Strain 1394N20 was cultured in de Man–Rogosa–Sharpe broth (Difco, Franklin Lakes, NJ, USA) at 35℃ for 24 h using the BD GasPak EZ Anaerobe Container System (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). Genomic DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), according the manufacturer's instructions. The complete genome of the strain was sequenced by DNALINK (Seoul, Korea) using PacBio RSII (Pacific Biosciences, Melon Park, CA, USA). These sequences were assembled *de novo* using the RS Hierarchical Genome Assembly Process version 3.0 [5]. The genomes of strain 1394N20 were annotated using Pathosystems Resource Integration Center (PATRIC) version 3.6.9 [6]. Functional annotation was performed using Kyoto Encyclopedia of Genes and Genomes database (www.genome.jp/kegg) [7] and evolutionary genealogy of genes: Non-supervised Orthologous Group-mapper version 2 (http://eggnog-mapper.embl.de) [8].

The genome sequences of the strain consisted of one chromosome, with a 581.2× sequencing depth (coverage). The quality parameters for the genome assembly, namely coarse consistency (99.4%) and fine consistency (97.6%), indicated the good quality of the assembled genome evaluated using PATRIC. The complete genome of strain 1394N20 was 2,176,326 bp long, with a guanine + cytosine content of 37.8 mol%. Moreover, the chromosome comprised of 2,281 protein-coding sequences, 15 rRNA genes (five 5S, five 16S, and five 23S), 3 ncRNA genes, and 65 tRNA genes (Table 1 and Fig. 1). The genome possessed *bcsZ* gene encoding endoglucanase. The protein encoded by this gene is decomposed by endohydrolysis of the D-glucosidic linkage of cellulose. In addition, it contained genes including *β*-glucosidase (*bglX*), 6-phospho-*β*-glucosidase (*bglA*), and cellobiose phosphotransferase system EIIA component (*celC* and *chbA*), which converts cellobiose into D-glucose. Based on this information, it is suggested that *L. amylovorus* strain 1394N20 can facilitate sugar absorption by decomposing cellulose, which is difficult to digest in the intestine.

Table 1. Genomic features of *Lactobacillus amylovorus* strain 1394N20

Properties	Value
BioProject	PRJNA726865
BioSample	SAMN18972587
Accession No.	CP074196
Sequencing method	PacBio RSII
Assembly method	HGAP v3.0
Genome size (bp)	2,176,326
Contig	1
Total CDSs	2,281
rRNA genes	15
tRNA genes	65
G + C content (mol%)	37.8

HGAP, hierarchical genome assembly process; CDSs, coding sequences; G + C, guanine + cytosine.
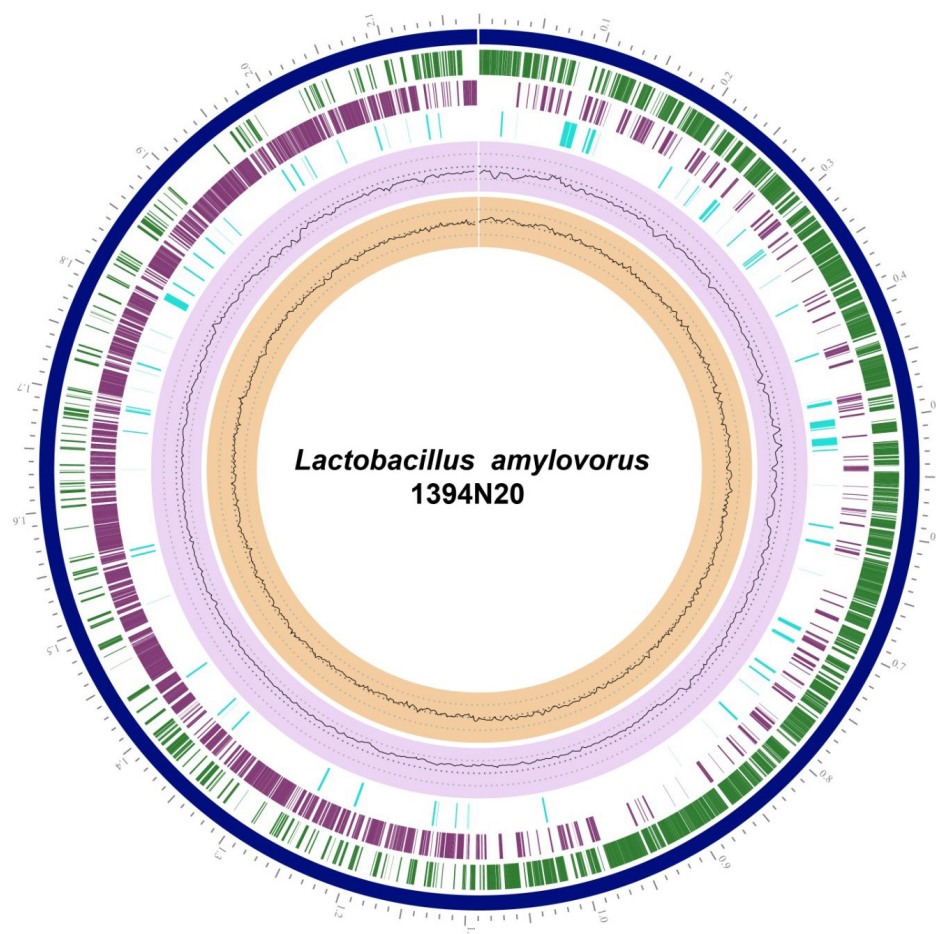
Fig. 1. Circular view of the genome of *Lactobacillus amylovorus* strain 1394N20 showing the physical map of its significant features generated using PATRIC. From outside to inside: contigs (blue), annotated reference genes (particularly, coding sequences [CDSs]) on the forward strand (green), and annotated reference genes on the reverse strand (purple). The fourth circle shows non-CDSs featured in the genome (light blue). The next circle indicates guanine–cytosine (GC) content (lavender/light purple), while the innermost circle indicates the GC skew (peach).

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The GenBank accession number for the genome of *L. amylovorus* strain 1394N20 is CP074196.

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