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Complete genome sequence of probiotic *Lactobacillus johnsonii* 7409N31 isolated from a healthy Hanwoo calf

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Abstract

Lactobacillus johnsonii 7409N31 was isolated from the feces of a healthy 11-day-old Hanwoo calf from a farm in Geochang-gun, Gyeongsangnam-do, Korea. The genome of the strain was completely sequenced using the PacBio RSII sequencing system, and it was confirmed that it was composed of one circular chromosome. The size of the entire genome was 2,198,442 bp, and it had 35.01 mol% guanine + cytosine (G + C) content and 2,222 protein-coding sequences, 24 rRNA, 3 ncRNA, and 112 tRNA genes. Strain 7409N31 possessed genes encoding enzymes involved in the hydrolysis of both fibrous and non-fibrous carbohydrates. These data provide a comprehensive theoretical understanding for developing industrial probiotic feed additives that improve nutrient digestibility.

Keywords: *Lactobacillus johnsonii*, Complete genome sequence, Hanwoo calf, Probiotics

Lactic acid bacteria (LAB) have been associated with the fermentation and preservation of food since ancient times and are one of the most important groups of industrial microorganisms with a multi-billion dollar market. They are naturally found in fermented foods as well as in human and animal cavities, including the gastrointestinal tract [1,2]. LAB colonizes the intestines of animals as a part of the normal intestinal flora, inhibit the growth of harmful bacteria, prevent diarrhea, and inhibit the absorption of toxic substances [3]. Ruminants utilize intestinal microbes to break down cellulose into glucose so that it can be used as an energy source, and some LAB are known to use cellulose as a carbon source [4]. Ruminant roughage contains both fibrous (cell wall material) and non-fibrous (cell content) carbohydrates, but most of them are composed of fibers such as cellulose, hemicellulose, and pectin [5].

In the present study, *Lactobacillus johnsonii* 7409N31 (KCCM 13026P) was isolated from the feces of a healthy 11-day-old Hanwoo calf. Strain 7409N31 was anaerobically grown in deMan, Rogosa, and Sharpe (MRS, Difco, Franklin Lakes, NJ, USA) medium at 35℃ for 24 h. Genomic DNA of

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Competing interests
No potential conflict of interest relevant to this article was reported.

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Not applicable.

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, Lee J, Park J, Choi HJ.
Data curation: Oh YJ, Lee J.
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Software: Oh YJ, Lim SK.
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Writing - review & editing: Oh YJ, Lee J, Lim SK, Kwon MS, Lee S, Choi SP, Yu D, Oh YS, 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.

7409N31 strain was extracted as described previously [6]. The complete genome of *L. johnsonii* 7409N31 was sequenced by using Pacific Biosciences (PacBio) reagents (DNA Link, Seoul, Korea). A total number of 735,552 reads with a mean subread length of 5,638 bases (N50, 6,459 bases) were obtained with PacBio sequencing. These sequences were assembled *de novo* using the Hierarchical Genome Assembly Process (HGAP, version 3.0) workflow [7]. The genome of the *L. johnsonii* 7409N31 was annotated using the NCBI Prokaryotic Genome Annotation Pipeline and the Pathosystems Resource Integratin Center (PATRIC, version 3.6.12) genome data base [8]. Genome assembly completeness was evaluated using Benchmarking Universal Single-Copy Orthologous suite (BUSCO, version 5.1.3) [9], and evolutionary genealogy of genes: Non-supervised Orthologous Group-mapper (EggNOG-mapper, version 2.0, http://eggnog-mapper.embl.de) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (www.genome.jp/kegg) were used for functional annotation. The complete genome of strain 7409N31 has one circular chromosome (2,198,442 bp), with a guanine + cytosine (G + C) content of 35.01 mol%, 2,222 protein-coding sequences, 24 rRNA, 3 ncRNA, and 112 tRNA genes (Fig. 1 and Table 1). Further analysis showed that about 80% of the protein-coding genes (1,778 proteins) could be clustered in 21 functional categories of cluster of orthologous groups (COG) (Fig. 2). Most of the proteins of strain 7409N31 were classified into categories related to 'core functions' such as replication, recombination and repair (category L: 10.2%), transcription (category K: 8.9%), translation, ribosomal structure and biogenesis (category J: 8.7%), and carbohydrate transport

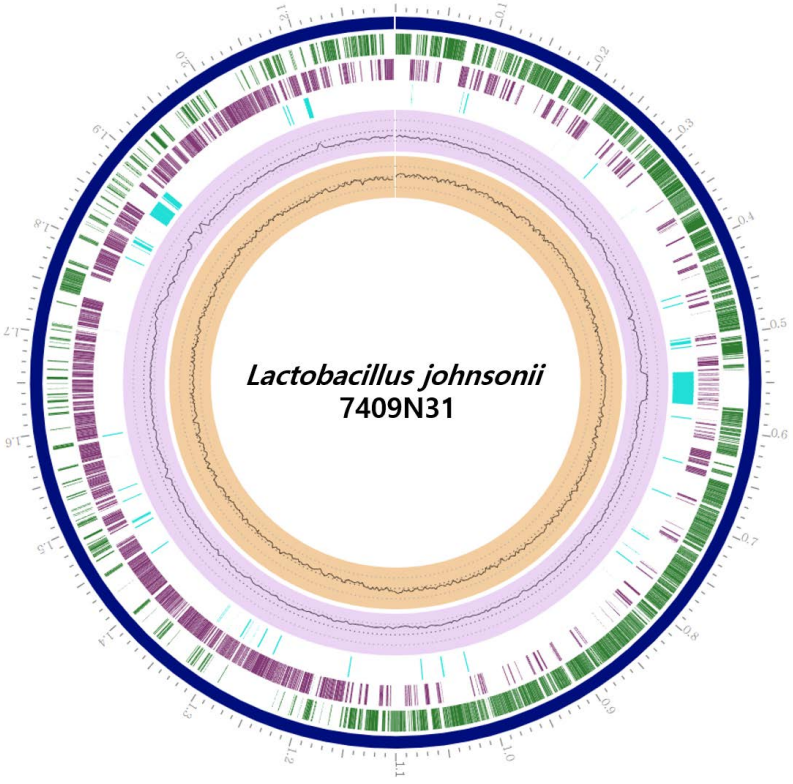

Fig. 1. Circular view of the genome of *Lactobacillus johnsonii* 7409N31 showing the physical map of its significant features generated using PATRIC. From outside to inside: contigs (blue), annotated reference genes (particularly, coding sequences [CDSs]) on the forward strand (green), and annotated 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).

Table 1. Genomic features of *Lactobacillus johnsonii* strain 7409N31

Properties	Value
BioProject	PRJNA766157
BioSample	SAMN21619988
Accession No.	CP084221
Sequencing method	PacBio RSII
Assembly method	HGAP version 3.0
Genome size (bp)	2,198,442
Contig	1
Total CDSs	2,222
rRNA genes	24
tRNA genes	112
G + C content (mol%)	35.01

HGAP, hierarchical genome assembly process; CDSs, coding sequences; G + C, guanine + cytosine.

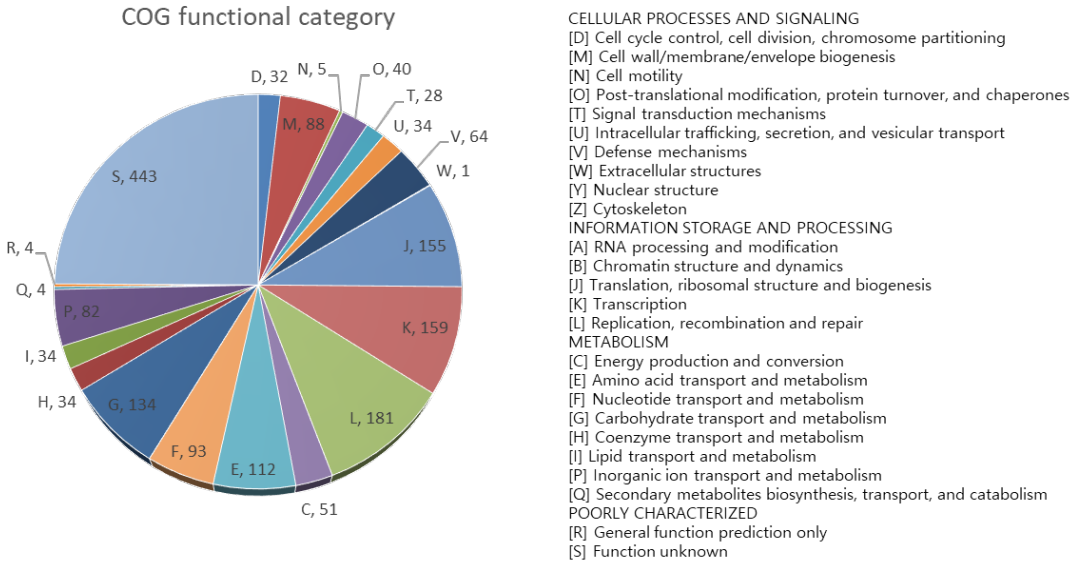

Fig. 2. **COG functional annotation of *Lactobacillus johnsonii* 7409N31.** COG, cluster of orthologous groups.

and metabolism (category G: 7.5%). This suggests that strain 7409N31 has a relatively strong carbohydrate metabolism ability similar to other strains in the genus *Lactobacillus* [10].

The genome of *L. johnsonii* 7409N31 possessed the *bcsZ* gene that encodes an endoglucanase involved in the hydrolysis of the D-glycosidic bond of cellulose. In addition, the presence of genes such as beta-fructofuranosidase (*sacA*), cellobiose PTS components (*celB* and *chbC*), and oligo-1,6-glucosidase (IMA, *malL*) involved in the metabolism of non-fibrous carbohydrates such as starch was confirmed. These results suggest that *L. johnsonii* strain 7409N31 has the potential to be developed as an industrial probiotic feed additive because it can facilitate digestion of both fibrous and non-fibrous carbohydrates.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The GenBank accession number for the genome of *L. johnsonii* strain 7409N31 is CP084221.

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