RESEARCH ARTICLE

J Anim Sci Technol 2026;68(1):366-370
https://doi.org/10.5187/jast.2024.e62

JAST
Journal of Animal Science and Technology
pISSN 2672-0191 eISSN 2055-0391

Complete genome sequence of bacteriocin-producing *Lactococcus lactis* subsp. *lactis* DOME 6301 with potential oral-pathogen control applications

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Received: Apr 11, 2024
Revised: Jun 10, 2024
Accepted: Jun 12, 2024

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Abstract

Lactococcus lactis subsp. *lactis* DOME 6301, isolated from bovine milk, produces an antimicrobial compound that inhibits oral pathogens including *Streptococcus mutans*, *Prevotella intermedia*, and *Fusobacterium nucleatum*. The entire genome of *L. lactis* DOME 6301 was sequenced and assembled *de novo* using the PacBio RS II platform. The genome was 2,532,858 bp in length, assembled into three contigs, and had a guanine and cytosine (G + C) ratio of 35%. The annotation results revealed 2,469 protein-coding sequences, 22 rRNA genes, and 78 tRNA genes. Genes involved in the utilization of complex carbohydrates (i.e., cellulose, xylose, pullulan, amylose, maltodextrin, and arabinofuranose) and synthesis of the bacteriocin, nisin Z, were detected. In addition, genes encoding antimicrobial resistance and virulence factors (i.e., hemolysin and enterotoxin) were detected. Whole-genome analysis of strain DOME 6301 contributed to our understanding of the evolution of the taxa and provided the basis for the correct selection of probiotic candidates for downstream applications.

Keywords: *Lactococcus lactis* subsp. *lactis*, Genome announcement, Nisin Z bacteriocin, Oral pathogens, Complex carbohydrates

Lactococcus lactis is a lactic acid bacterium (LAB) that has been designated by the United States Food and Drug Administration as generally recognized as safe. *Lactococcus lactis* subsp. *lactis* is frequently present in naturally fermented dairy products and is widely employed in commercial feed, milk fermentation, and vaccine manufacturing [1]. However, several studies have indicated that *L. lactis* can cause mastitis in cows and it has even been associated with clinical cases (e.g., lactococcosis in silver carp, liver and spleen disease in waterfowl, and endocarditis in humans). A functional genomic study revealed that dairy *L. lactis* subsp. *lactis* diverged from plant-associated ancestors independently of human intervention, but was later selected for its functional properties in dairy fermentation [2].

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

Funding sources
This research was supported by the Chung-Ang University Graduate Research Scholarship in 2022.

Acknowledgements
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: Huh CS, Kim GB.
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Formal analysis: Elnar AG, Jang Y, Huh CS, Kim GB.
Methodology: Elnar AG, Jang Y, Eum BG, Hur Y, Huh CS, Kim GB.
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Investigation: Elnar AG, Jang Y, Eum BG, Hur Y.
Writing - original draft: Elnar AG, Jang Y.
Writing - review & editing: Elnar AG, Jang Y, Eum BG, Hur Y, Huh CS, Kim GB.

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

Selection pressure leads to a reduction in the genome and the loss of genes, while acquiring genes involved in protein and lactose metabolism through horizontal gene transfer (HGT) [3]. HGT is also believed to be the main reason for the transmission of genes for antimicrobial resistance (i.e., erythromycin) and virulence factors from other species, conferring harmful traits to *L. lactis* subsp. *lactis* [4]. Further efforts are required to fully understand the evolutionary divergence of the group and to better understand the differences between safe and potentially pathogenic strains of *L. lactis*.

Here, we present the whole genome of *Lactococcus lactis* subsp. *lactis* DOME 6301 strain, which has the ability to produce antimicrobial compounds, isolated from raw cow milk. Strain DOME 6301 was routinely cultured in de Man, Rogosa, and Sharpe (BD Difco) broth supplemented with 0.05% L-cysteine HCl (Sigma Aldrich). Genomic DNA was extracted from 12–15 h cultures using the QIAamp PowerFecal DNA Kit (Qiagen) following the prescribed protocol. Sequencing was performed at CJ Bioscience using the Pacific Biosciences RSII Single Molecule Real-Time platform with a 20-kb SMRTbell^{TM} template library (PacBio), followed by *de novo* assembly of the reads using FALCON 0.5. Whole-genome analysis of *L. lactis* subsp. *lactis* DOME 6301 (Fig. 1) revealed a genome of 2,532,858 base pairs with a guanine and cytosine content (G + C content) of 35.0% and an N_{50} value of 2,417,727 bp, assembled into three contigs, one of which was designated as plasmid pDOME6301-LcnB (103,795 bp). The genome consisted of 2,469 protein-coding genes, 78 tRNA genes, and 22 rRNA genes (Table 1). Genome annotation and functional categorization were performed using Rapid Annotation Subsystem Technology (http://rast.nmpdr.org/) with default parameters, and a cluster of orthologous groups was obtained from the EZBioCloud server. As shown in Fig. 2, most genes were predicted to be involved in cell wall and membrane envelope biogenesis (121); translation, ribosomal structure, and biogenesis (156); amino acid transport and metabolism (190); carbohydrate transport and metabolism (182); and inorganic ion transport and metabolism (128).

Among the predicted carbohydrate-related genes, several genes encoding enzymes for complex carbohydrate utilization were found, including cellulase, endo-1,4-β-xylanase, oligosaccharide reducing-end xylanase, diamine N-acetyltransferase, α-amylase, cyclomaltodextrinase, pullulanase, non-reducing-end α-L-arabinofuranosidase, and an uncharacterized multiple-sugar transport system permease YteP, which may hold significant function in carbohydrate utilization in the animal host. Additionally, two bacteriocin gene clusters corresponding to nisin Z (Class I; chromosomally encoded) and lactococcin B (Class IID; plasmid-encoded) were identified using the BAGEL4 webserver (http://bagel4.molgenrug.nl/) as depicted in Fig. 1C and D. Downstream the nisin Z open reading frame (ORF) contained genes for bacteriocin modification (*lanB* and *lanC*), regulation (*lanR* and *lanK*), immunity and transport (*nisT*, *nisF*, and nisin immunity proteins), and a serine protease for leader peptide cleavage. The lactococcin B operon contained an ORF for the core peptide and an immunity protein. Preliminary experiments showed that the bacteriocins produced by strain DOME 6301 inhibited the growth of oral pathogens, including *Streptococcus mutans* KCTC 5365, *Prevotella intermedia* KCTC 15693^{T}, and *Fusobacterium nucleatum* KCTC 2488^{T}, implying that this strain could be used as a probiotic candidate for the development of functional dairy products having antimicrobial properties.

On an evolutionary level, the strains are thought to differ based on their carbohydrate metabolism, ability to defend themselves by producing antimicrobial compounds, and how they react to stress [5]. Antimicrobial resistance genes were predicted using the Comprehensive Antibiotic Resistance Database Resistance Gene Identifier [6], which revealed the presence of *vanY* (% ID, 33.7%) and *qacJ* (% ID, 46.67%) genes associated with resistance to glycopeptide antibiotics and disinfecting agents or antiseptics, respectively. Furthermore, genes encoding virulence factors, including hemolysin (hemolysin-3, a conserved virulence factor) and enterotoxin were detected in

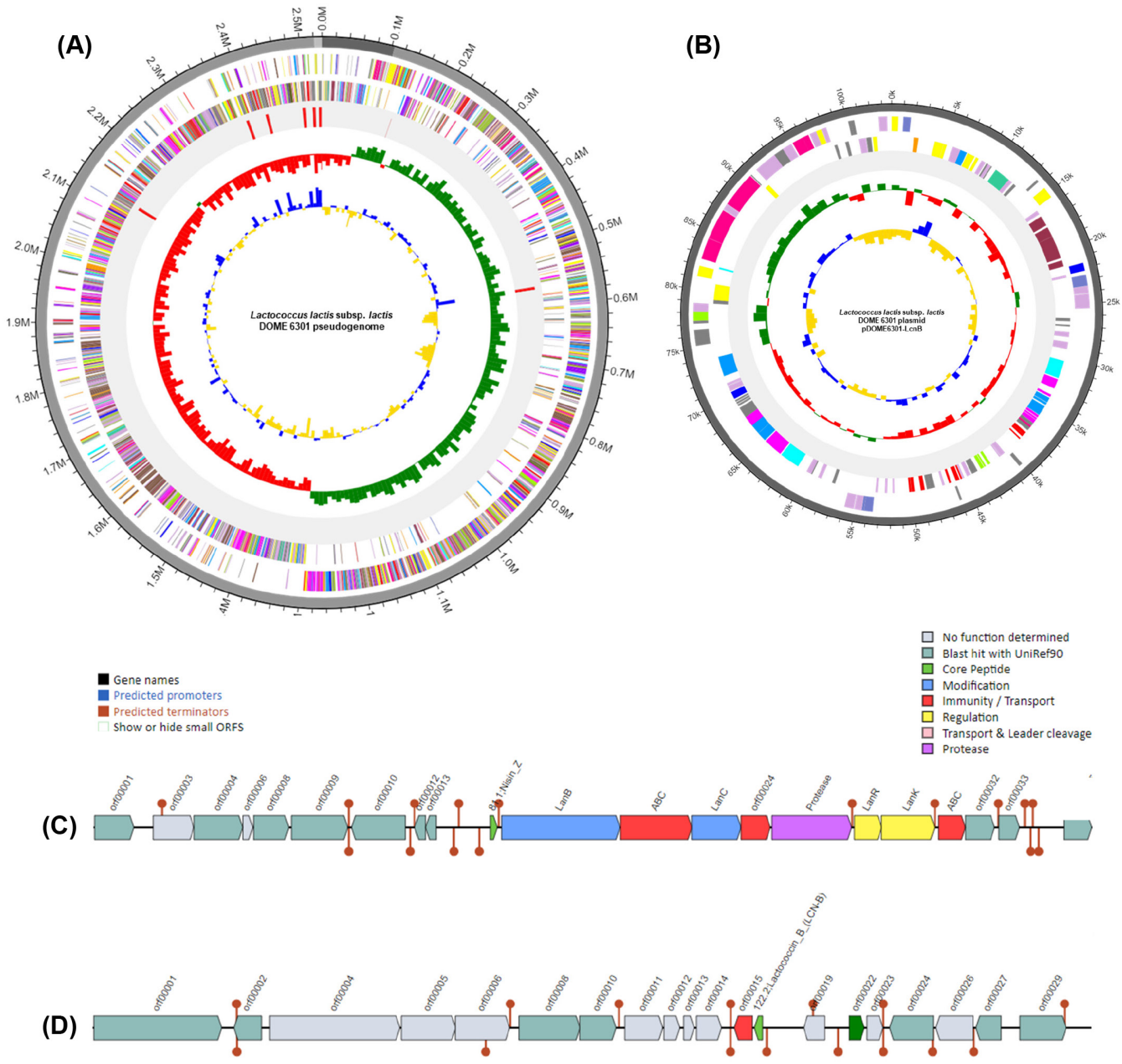

Fig. 1. Circular genome maps of *Lactococcus lactis* subsp. *lactis* DOME 6301 (A) pseudogenome and (B) plasmid pDOME6301-LcnB. Circles represent the following characteristics from the outermost circle to the center: (1) contig information, (2) coding sequences on forward strand, (3) coding sequences on reverse strand, (4) transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), (5) GC skew, and (6) GC ratio. Coding sequences and predicted bacteriocin gene cluster in *Lactococcus lactis* subsp. *lactis* DOME 6301 genome for (C) Nisin Z and (D) Lactococcin B. G, guanine; C, cytosine; CDS, coding sequences.

the chromosome (Supplementary Information). Despite the significant potential of strain DOME 6301 in various industrial applications owing to the presence of enzymes for the breakdown of complex carbohydrates, the presence of genes involved in hemolytic activity and enterotoxins might limit its potential use. Nevertheless, nisin Z, a structural variant of the commercially accepted nisin A (His27 > Asn), remains valuable for pathogen control and is a possible alternative to conventional antimicrobials [7]. These observations contribute to the elucidation of the evolutionary background

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Table 1. Genome characteristics of *Lactococcus lactis* subsp. *lactis* DOME 6301

Attribute	Value
Genome size (bp)	2,532,858
GC content (%)	35.0
No. of contigs	3
Total genes	2,569
Protein-coding genes	2,469
tRNA genes	78
rRNA genes	22
Plasmids	1
GenBank Accession No.	JBBVGU000000000

G, guanine; C, cytosine.

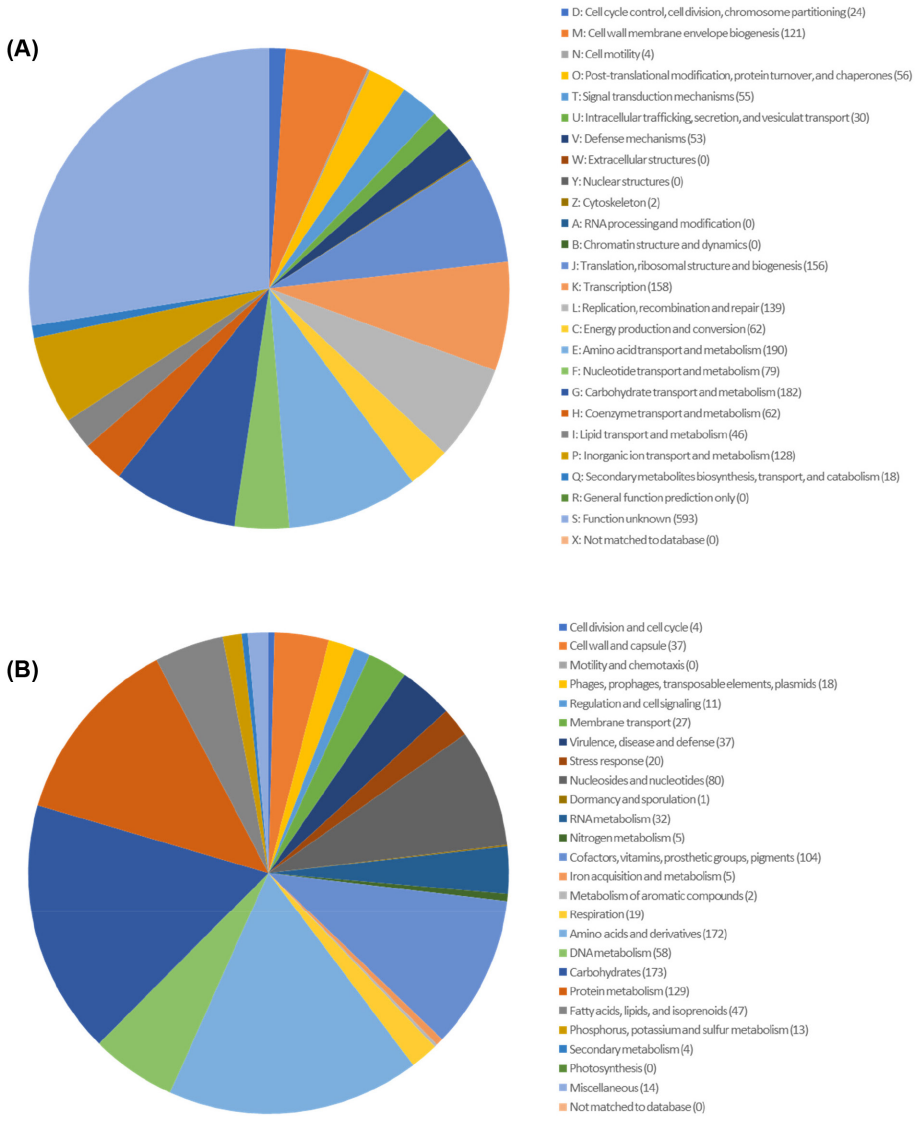

Fig. 2. Distribution by Cluster of Orthologous Group (A) and KEGG annotation (B) based on the functional classification of whole genome of *Lactococcus lactis* subsp. *lactis* DOME 6301. KEGG, Kyoto Encyclopedia of Genes and Genomes.

of *L. lactis* subsp. *lactis* and highlight the importance of intensive and accurate characterization of LAB strains for their potential use in the fermentation industry or for the development of functional probiotics.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The sequence obtained in this whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under accession number JBBVGU000000000. The BioProject accession number is PRJNA1095286 and the Biosample accession number is SAMN40716561.

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