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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Genome Announcement
Article Title (within 20 words without abbreviations)	Complete genome sequence of bacteriocin-producing *Lactococcus lactis* subsp. *lactis* DOME 6301 with potential oral-pathogen control applications
Running Title (within 10 words)	Complete genome sequence of *Lactococcus lactis* subsp. *lactis* DOME 6301
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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

Main Text

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]. 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, Sparks, MD, USA) broth supplemented with 0.05% L-cysteine HCl (Sigma Aldrich, St Louis, MO, USA). Genomic DNA was extracted from 12–15 h cultures using the QIAamp PowerFecal DNA Kit (Qiagen, Hilden, Germany) following the prescribed protocol. Sequencing was performed at CJ Bioscience (Seoul, Korea) using the Pacific Biosciences RSII Single Molecule Real-Time platform with a 20-kb SMRTbell^{TM} template library (PacBio, Menlo Park, CA, USA), 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-ß-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. 3. 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* KCTC5365, *Prevotella intermedia* KCTC 15693^T, and *Fusobacterium nucleatum* KCTC 2488^T, implies 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 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 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 JBBVGU10000000. The BioProject accession number is PRJNA1095286 and the Biosample accession number is SAMN40716561.

Acknowledgments

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References

1. Wu F, Xie X, Du T, Jiang X, Miao W, Wang T (2023) Lactococcus lactis, a bacterium with probiotic functions and pathogenicity. World J Microbiol Biotechnol 39(12):325. https://doi.org/10.1007/s11274-023-03771-5

2. Liu W, Li W, Zheng H, Kwok LY, Sun Z (2022) Genomics divergence of Lactococcus lactis subsp. lactis isolated from naturally fermented dairy products. Food Res Int 155:111108. https://doi.org/10.1016/j.foodres.2022.111108

3. Cavanagh D, Fitzgerald GF, McAuliffe O (2015) From field to fermentation: the origins of Lactococcus lactis and its domestication to the dairy environment. Food Microbiol 47:45-61. https://doi.org/10.1016/j.fm.2014.11.001

4. Colautti A, Arnoldi M, Comi G, Iacumin L (2022) Antibiotic resistance and virulence factors in lactobacilli: something to carefully consider. Food Microbiol 103:103934. https://doi.org/10.1016/j.fm.2021.103934

5. Nguyen TL, Kim DH (2018) Genome-Wide Comparison Reveals a Probiotic Strain Lactococcus Lactis WFLU12 Isolated from the Gastrointestinal Tract of Olive Flounder (Paralichthys Olivaceus) Harboring Genes Supporting Probiotic Action. Mar Drugs 16(5). https://doi.org/10.3390/md16050140

6. Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, et al. (2023) CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic Acids Res 51(D1):D690-D9. https://doi.org/10.1093/nar/gkac920

7. Malaczewska J, Kaczorek-Lukowska E (2021) Nisin-A lantibiotic with immunomodulatory properties: A review. Peptides 137:170479. https://doi.org/10.1016/j.peptides.2020.170479

Legends of Tables and Figures

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	0
GenBank Accession No.	TBA

G, guanine; C, cytosine.

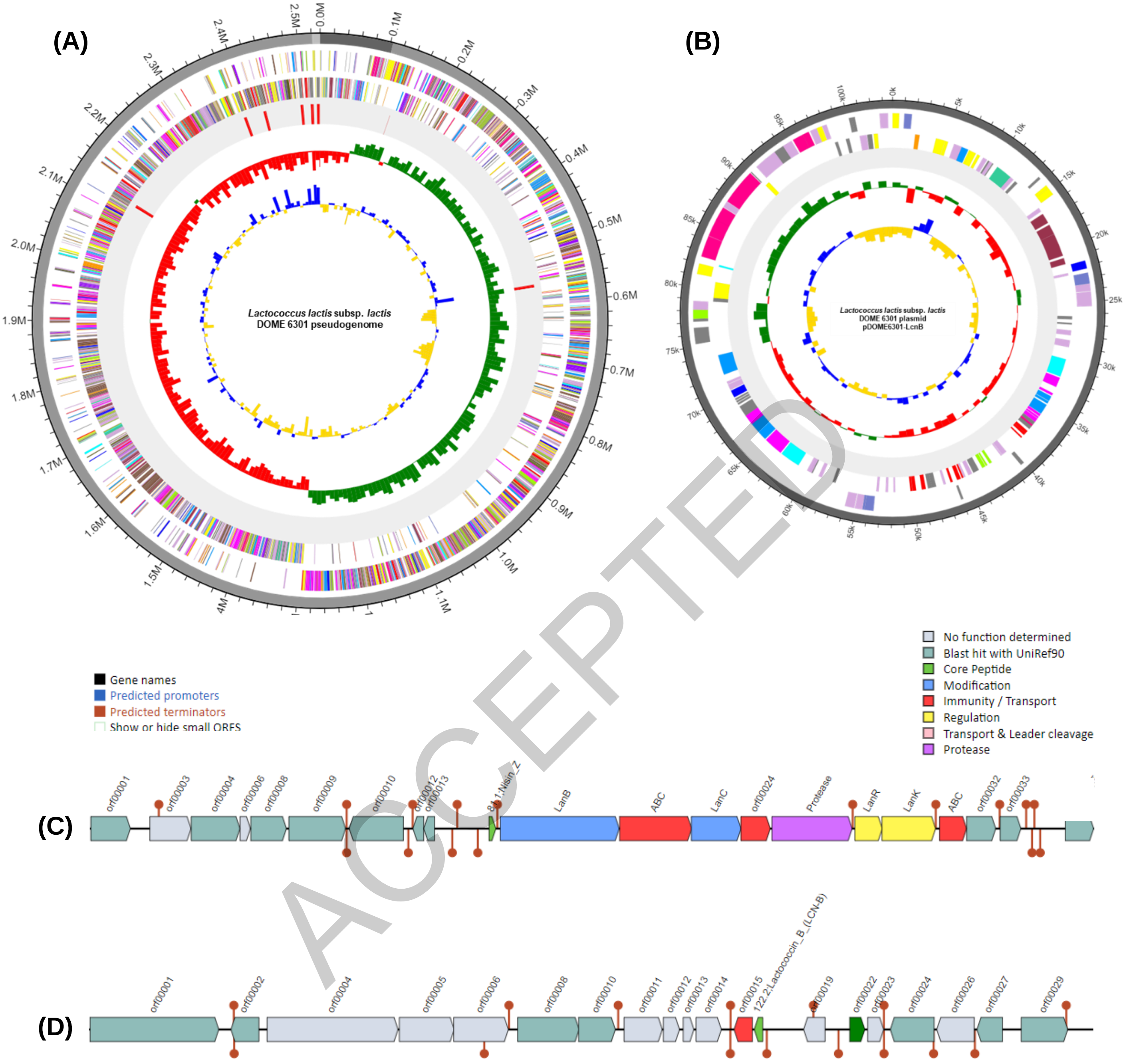

Fig. 1. Circular genome maps of *Lactococcus lactis* subsp. *lactis* DOME 6301 (A) pseudogenome and (B) plasmid pDOME6301-LcnB. Circles represent the following characteristics from the outermost circle to the center: (1) contig information, (2) coding sequences on forward strand, (3) coding sequences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

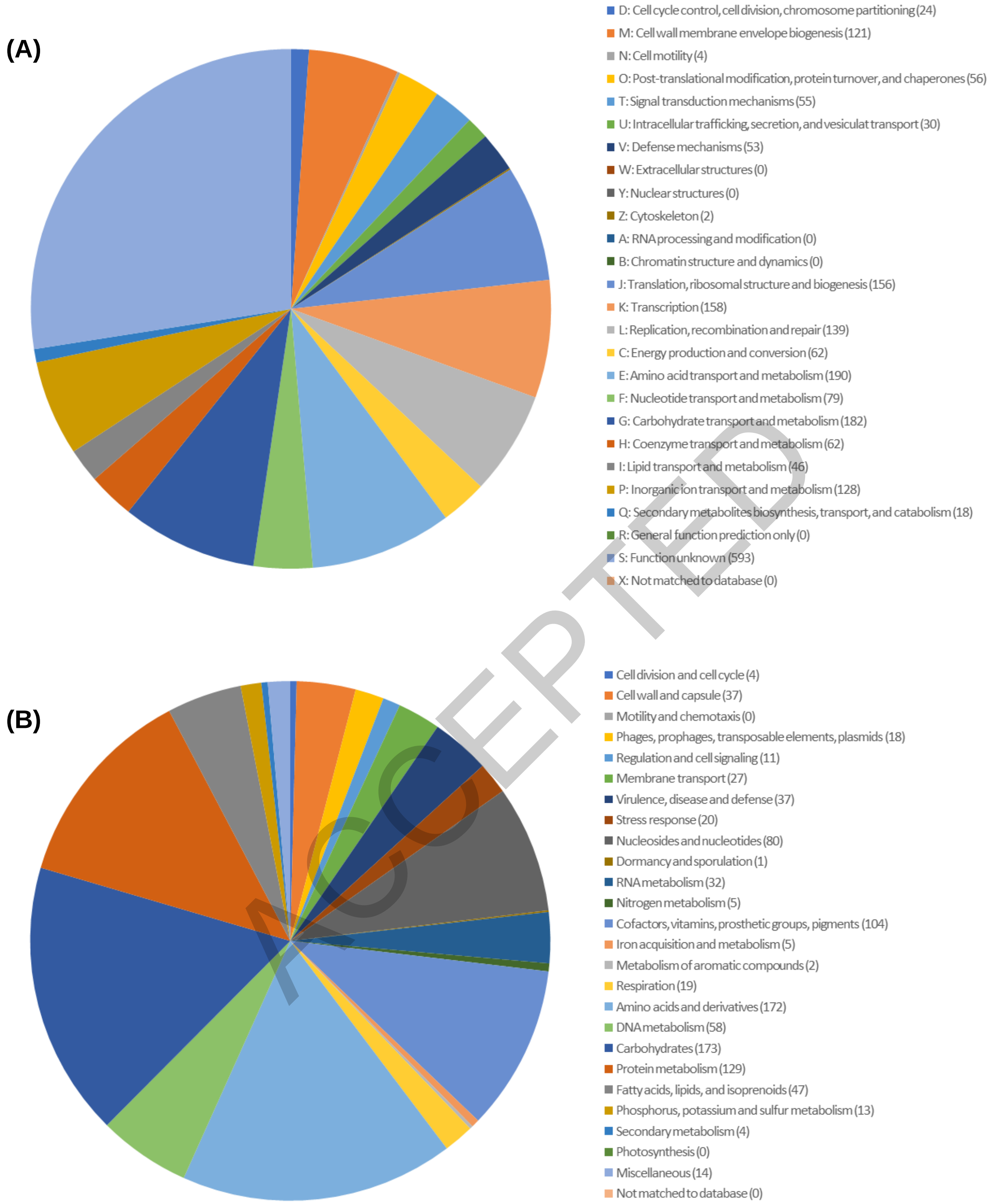

Fig. 2. Distribution by KEGG annotation (A) and Cluster of Orthologous Group (B) based on the functional classification of whole genome of *Lactococcus lactis* subsp. *lactis* DOME 6301.

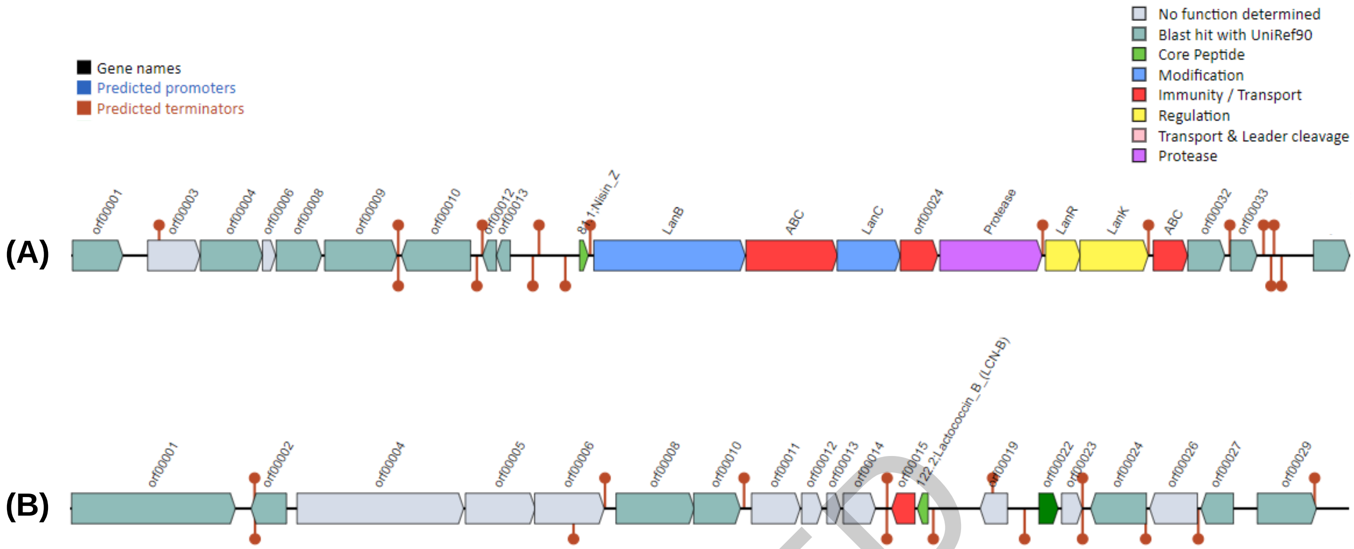

Fig. 3. Predicted bacteriocin gene clusters in *Lactococcus lactis* subsp. *lactis* DOME 6301 genome for (A) Nisin Z and (B) Lactococcin B.