RESEARCH ARTICLE

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Complete genome sequence of potential probiotic *Ligilactobacillus ruminis* CACC881 isolated from swine

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

Abstract

Ligilactobacillus ruminis is a gram-positive anaerobic bacterium in the host intestinal tract. *L. ruminis* has not been extensively studied, resulting in limited data regarding its potential probiotic properties and genomic information. In this study, the genome of *L. ruminis* CACC881 was comprehensively analyzed, resulting in the prediction of potential probiotic characteristics. Additionally, a comparative genomic analysis was conducted on the five *L. ruminis* strains. The genome of strain CACC881 comprised one circular chromosome 2,107,343 bp in length. Among the predicted 1,935 protein-coding genes, the genome included genes associated with potential probiotic properties, such as acid/bile salt tolerance, clustered regularly interspaced short palindromic repeats (CRISPR)-related genes, and vitamin B-group genes. Notably, the genes for bacteriocin regulation/immunity (*nisK* and *nisI*) and antioxidant activity (*ahpC*) were exclusively found in strain CACC881, while absent in the other four *L. ruminis* genomes. These findings suggest that *L. ruminis* CACC881 is a potential probiotic with applications for the animal industry.

Keywords: *Ligilactobacillus ruminis*, Swine, Probiotics, PacBio, Genome sequence

ANNOUNCEMENT

Probiotics are known to contribute to animal intestinal health, performance, and productivity [1]. They are widely used as a food additive in the animal industry. When selecting a probiotic strain, the strain should have acid/bile salt tolerance and cell-adhesion abilities for intestinal survival, as well as other functional properties, including immunomodulatory, antimicrobial, and antioxidant abilities [2,3].

Ligilactobacillus ruminis is an anaerobic, gram-positive bacteria that is autochthonous in the gastrointestinal tract of many animals. It is a lactic acid bacteria found in the large intestine of swine [4]. *L. ruminis* reportedly has an immunomodulatory effect and can suppress pathogens in the host [3]. However, few studies have documented the potential probiotics properties of *L. ruminis*. In this

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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: Kim Y.
Data curation: Park S, Park MA.
Formal analysis: Park S.
Methodology: Park S, Jang HJ.
Validation: Park S, Kim DH.
Investigation: Park S.
Writing - original draft: Park S.
Writing - review & editing: Park S, Park MA, Jang HJ, Kim DH, Kim Y.

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

study, the genome of *L. ruminis* CACC881 was analyzed for its potential probiotic properties, and a comparative genomic analysis was performed on four other *L. ruminis* strains. Fecal samples from 30 days old weaned pigs (Duroc × Landrace × Yorkshire) were collected from a pig farm in Jeongeup City in North Jeolla Province, Korea. The samples were serially diluted with sterile saline buffer and cultured on de Man, Rogosa, and Sharpe (MRS; Merck KGaA) medium under anaerobic conditions at 37℃ for 24 h. After randomly selecting single colonies, they were transferred onto MRS medium for further culturing. Identification was conducted through 16S rRNA sequencing, employing the primers 518F (5'-CCAGCAGCCGCGGTAATAC-3') and 805R (5'-GACTACCAGGGTATCTAATC-3'). After identifying *L. ruminis* CACC881 (KCTC 25583) from the cultured colony, whole-genome sequencing was conducted. Genomic DNA was extracted from *L. ruminis* CACC881 cultured in MRS medium at 37℃ for 24 h, using the UltraClean microbial kit (Qiagen), and then sequenced on the PacBio Sequel II platform (Pacific Biosciences) for whole-genome analysis. The sequenced raw data were assembled using PacBio SMRT analysis software (version 2.3.0, Pacific Biosciences) [5]. Protein-coding sequences (CDSs) were predicted using the Prodigal 2.6.2 program built into the EzBioCloud server, and the information was validated using the National Center for Biotechnology Information blast. Additionally, bacteriocin-related genes were identified using the BAGEL 4.0 web software (http://bagel5.molgenrug.nl/). The genes were functionally annotated using clusters of orthologous group (COG)-based EggNOG and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases [5]. The orthologous average nucleotide identity (OrthoANI) value of the CACC881 strain was compared with that of related strains (ATCC25644, ATCC27780, PEL65, and DSM20403). A heatmap of the OrthoANI values was constructed using the OrthoANI Tool on the EzBioCloud server. Pan-genome orthologs (POGs) were analyzed using UBLAST with an *E*-value threshold of 10^{-6} [2]. A Venn diagram of the calculated POGs was constructed using the Venn program [6].

The complete genome of *L. ruminis* CACC881 comprised one circular chromosome (2,107,343 bp) with a GC content of 43.4%, 1,935 predicted CDSs, and 85 non-coding genes (19 rRNA and 66 tRNA genes) (Fig. 1A). In total, 1,790 CDSs (92.5%) were functionally classified into 19 COG categories (Fig. 1B). Most of the known protein-coding genes were associated with replication/recombination/repair (12.0%), amino acid transport and metabolism (8.4%), translation/ribosomal structure/biogenesis (8.0%), carbohydrate transport and metabolism (5.9%), and transcription (5.9%). Among the *L. ruminis* strains, the complete genome of CACC881 was most similar to that of strains DSM20403 (97.3%) and ATCC25644 (97.3%) (Fig. 1C). The pan-genomes of CACC881 and its related strains contained 2,480 POGs, of which 1,523 (61.4%) were core groups shared by all five strains. In total, 219 POGs (8.8%) were unique to the CACC881 strain, which included 60 genes encoding hypothetical proteins (Table 1). The most abundant COG categories in strain-specific orthologs were replication/recombination/repair (16.0%), cell wall/membrane/envelope biogenesis (5.0%), and transcription (4.6%). The genome of the strain CACC881 encoded eight clustered regularly interspaced short palindromic repeats (CRISPR)-related genes/proteins, two immunomodulatory-related genes (*tagF* and *dltA*) [6], two antioxidant genes (*bcp* and *ahpC*) [7], and genes related to the biosynthesis of vitamin B groups (*ribT*, *ribF*, *frdA*, *ribU*, *ybjI*, and *cobC*/*phpB*) [8]. Additionally, Class I bacteriocin-related genes were observed, including those related to the regulation of nisin biosynthesis (*nisK*), nisin immunity (*nisI*), and lanthipeptide biosynthesis (*PSR47_00020*) [9]. The findings indicate that the strain CACC881 contained genes related to probiotic characterization, such as tolerance to acid (*clpB*, *queA*, and *grpE*) and bile salts (*cbh*), and lactate synthesis (*ldh*) [10]. Notably, genes related to the regulation/immunity of bacteriocin (*nisK* and *nisI*) and peroxiredoxin (*ahpC*) were found only in the genome of strain CACC881, and not in the genomes of the other four *L. ruminis* strains (Table 2). These findings predict that *L.*

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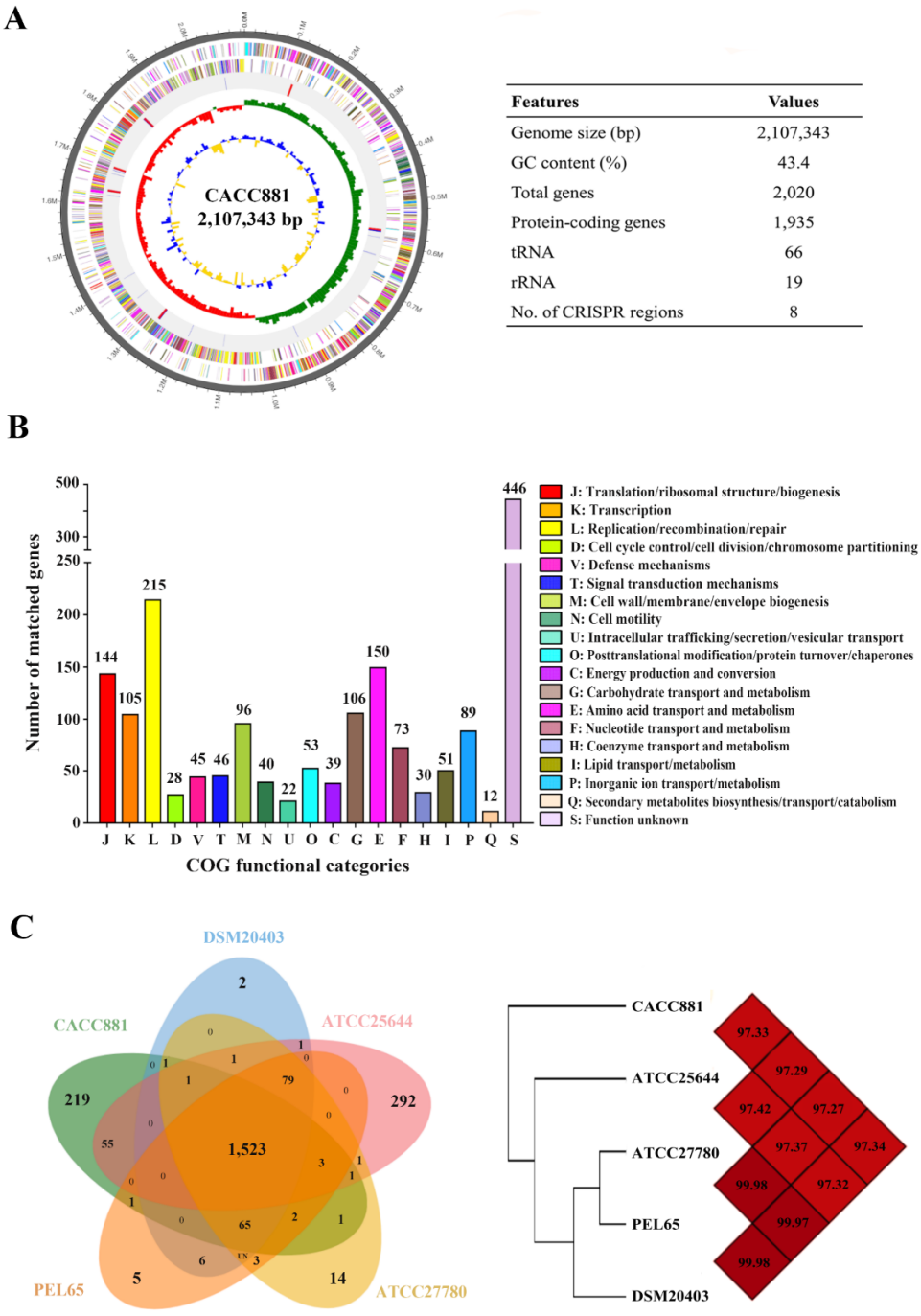

Fig. 1. Genomic features of *Ligilactobacillus ruminis* CACC881. (A) Circular genomic representation and features of *L. ruminis* CACC881. (B) Functional gene number of clusters of orthologous groups (COGs) categories. (C) Heatmap of orthologous average nucleotide identity (OrthoANI) and Venn diagram analysis of the genomes of five *L. ruminis* strains.

ruminis CACC881 will play a role as potential probiotic including characterization as bacteriocin, biosynthesis of vitamin B group, antioxidant, and immunomodulatory abilities, and possible contributions to gut health and pathogen protection. The complete genome sequence of *L. ruminis* CACC881 may also contribute to the understanding of probiotic characterization and possible

Table 1. Core and strain-specific gene clusters of five *Ligilactobacillus ruminis* strains

COG categories	Core POGs	CACC881-specific POGs
Unassigned	53	49
J	144	5
K	105	10
L	215	35
D	28	3
V	45	8
T	46	3
M	96	11
N	40	0
U	22	0
O	53	5
C	39	1
G	106	0
E	150	8
F	73	2
H	30	2
I	51	3
P	89	8
Q	12	6
S	446	60

COG, Clusters of Orthologous Groups; POG, pan-genome orthologous group; J, translation, ribosomal structure, and biogenesis; K, transcription; L, replication, recombination, and repair; D, cell cycle control, cell division, and chromosome partitioning; V, defense mechanisms; T, signal transduction mechanisms; M, cell wall/membrane/envelope biogenesis; N, cell motility; U, intracellular trafficking, secretion, and vesicular transport; O, posttranslational modification, protein turnover, chaperones; C, energy production and conversion; G, carbohydrate transport and metabolism; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; H, coenzyme transport and metabolism; I, lipid transport and metabolism; P, inorganic ion transport and metabolism; Q, secondary metabolites biosynthesis, transport, and catabolism; S, function unknown.

probiotic functions in animals.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The complete genome sequence of *L. ruminis* CACC881 has been deposited in GenBank under the accession number CP117687. The BioProject and accession numbers are PRJNA932598 and SAMN33198791, respectively.

REFERENCES

1. Vieco-Saiz N, Belguesmia Y, Raspoet R, Auclair E, Gancel F, Kempf I, et al. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Front Microbiol. 2019;10:57. https://doi.org/10.3389/fmicb.2019.00057
2. Oh YJ, Kim SA, Yang SH, Kim DH, Cheng YY, Kang JI, et al. Integrated genome-based assessment of safety and probiotic characteristics of Lactiplantibacillus plantarum PMO 08 isolated from kimchi. PLOS ONE. 2022;17:e0273986. https://doi.org/10.1371/journal.pone.0273986

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Table 2. **Predicted genes involved in the potential probiotic properties of *Ligilactobacillus ruminis* CACC881**

Predicted function	Gene	Start	End	Length (bp)
CRISPR-associated				
Endoribonuclease Cas1	*cas1*	c1,059,228	c1,060,136	909
Endonuclease Cas1	*cas1*	c1,089,938	c1,090,957	1,020
Endonuclease Cas2	*cas2*	c1,058,923	c1,059,225	303
Endoribonuclease Cas2	*cas2*	c1,089,635	c1,089,925	291
Nuclease/helicase Cas3	*cas3*	c1,095,199	c1,097,655	2,457
Endoribonuclease Cas6	*cas6*	c1,065,462	c1,066,229	768
Protein Cas10/Csm1	*cas10/csm1*	c1,063,199	c1,065,481	2,283
Type III-associated RAMP protein Csm3	*csm3*	c1,062,079	c1,062,747	669
Bacteriocin-related				
Lanthipeptides B (S8 family serine peptidase)	*PSR47_00020*	2,678	7,891	5,214
Regulation of nisin biosynthesis	*nisK*	c1,413,141	c1,414,505	1,365
Immunity	*nisI*	c1,415,215	c1,416,030	816
Lactate synthesis				
L-lactate dehydrogenase	*ldh*	1,736,388	1,737,359	972
	ldh	1,765,253	1,766,170	918
	ldh	c1,995,134	c1,996,102	969
Acid tolerance				
Chaperone protein ClpB	*clpB*	c1,108,538	c1,111,144	2,607
Chaperone protein GrpE	*grpE*	939,031	939,609	579
S-adenosylmethionine	*queA*	604,283	605,326	1,044
Bile salt tolerance				
Choloylglycine hydrolase	*cbh*	305,462	306,397	936
	cbh	1,010,862	1,011,215	354
	cbh	c1,914,050	c1,915,024	975
Stress response or protection				
Chaperone protein DnaK	*dnaK*	939,645	941,507	1,863
Chaperone protein DnaJ	*dnaJ*	941,609	942,745	1,137
Triose-phosphate isomerase	*tpiA*	c1,343,674	c1,344,429	756
Biosynthesis of vitamin B groups				
Riboflavin biosynthesis	*ribT*	819,421	819,801	381
Succinate dehydrogenase flavoprotein subunit	*frdA*	c16,031	c16,888	858
Riboflavin transporter RibU	*ribU*	822,069	822,743	675
FMN hydrolase	*ybjI*	1,012,073	1,012,870	798
Riboflavin kinase/FMN adenylyltransferase	*ribF*	c1,071,835	c1,072,794	369
Cobalamin biosynthesis	*cobC/phpB*	643,283	643,957	675
Key immunomodulatory molecule				
Teichoic acid biosynthesis	*tagF*	255,008	256,024	1,017
D-alanine--poly(phosphoribitol) ligase subunit 1	*dltA*	314,560	316,080	1,521
Antioxidant-associated	*tpx*	565,480	565,974	495
Thioredoxin-dependent peroxiredoxin	*bcp*	238,172	238,660	489
Peroxiredoxin	*ahpC*	c1,499,117	c1,499,680	324

CRISPR, clustered regularly interspaced short palindromic repeats.

3. Yu X, Åvall-Jääskeläinen S, Koort J, Lindholm A, Rintahaka J, von Ossowski I, et al. A comparative characterization of different host-sourced Lactobacillus ruminis strains and their adhesive, inhibitory, and immunomodulating functions. Front Microbiol. 2017;8:657. https://doi.org/10.3389/fmicb.2017.00657
4. Al Jassim RAM. Lactobacillus ruminis is a predominant lactic acid producing bacterium in the caecum and rectum of the pig. Lett Appl Microbiol. 2003;37:213-7. https://doi.org/10.1046/j.1472-765X.2003.01380.x
5. Park S, Kim JA, Jang HJ, Kim DH, Kim Y. Complete genome sequence of functional probiotic candidate Lactobacillus amylovorus CACC736. J Anim Sci Technol. 2023;65:473-7. https://doi.org/10.5187/jast.2022.e85
6. LeBlanc JG, Laiño JE, Del Valle MJ, Vannini V, van Sinderen D, Taranto MP, et al. B-Group vitamin production by lactic acid bacteria – current knowledge and potential applications. J Appl Microbiol. 2011;111:1297-309. https://doi.org/10.1111/j.1365-2672.2011.05157.x
7. Averina OV, Poluektova EU, Marsova MV, Danilenko VN. Biomarkers and utility of the antioxidant potential of probiotic Lactobacilli and Bifidobacteria as representatives of the human gut microbiota. Biomedicines. 2021;9:1340. https://doi.org/10.3390/biomedicines9101340
8. Rodrigo-Torres L, Yépez A, Aznar R, Arahal DR. Genomic insights into five strains of Lactobacillus plantarum with biotechnological potential isolated from chicha, a traditional maize-based fermented beverage from Northwestern Argentina. Front Microbiol. 2019;10:2232. https://doi.org/10.3389/fmicb.2019.02232
9. van Staden ADP, van Zyl WF, Trindade M, Dicks LMT, Smith C. Therapeutic application of lantibiotics and other lanthipeptides: old and new findings. Appl Environ Microbiol. 2021;87:e00186-21. https://doi.org/10.1128/AEM.00186-21
10. Wang G, Yu H, Feng X, Tang H, Xiong Z, Xia Y, et al. Specific bile salt hydrolase genes in Lactobacillus plantarum AR113 and relationship with bile salt resistance. LWT-Food Sci Technol. 2021;145:111208. https://doi.org/10.1016/j.lwt.2021.111208