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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, 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 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, Jeonbuk State, South Korea. The samples were serially diluted with sterile saline buffer and cultured on de Man, Rogosa, and Sharpe (MRS; Merck KGaA, Darmstadt, Germany) 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, Hilden, Germany), and then sequenced on the PacBio Sequel II platform (Pacific Biosciences, Menlo Park, CA, USA) 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, 1,750 CDSs (90.4 %) were functionally classified into 19 COG categories (Fig. 1B). Most of the known protein-coding genes were associated with replication/recombination/repair (12.3%), amino acid transport and metabolism (8.6%), transcription/ribosomal structure/biogenesis (8.2%), carbohydrate transport and metabolism (6.1%), and transcription (6.0%). 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 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*) (Table 2) [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. These findings predict that *L. 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 probiotic functions in animals.

Nucleotide sequence accession number(s)

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.

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Tables and Figures

Table 1. Core and strain-specific gene clusters of five *L. 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

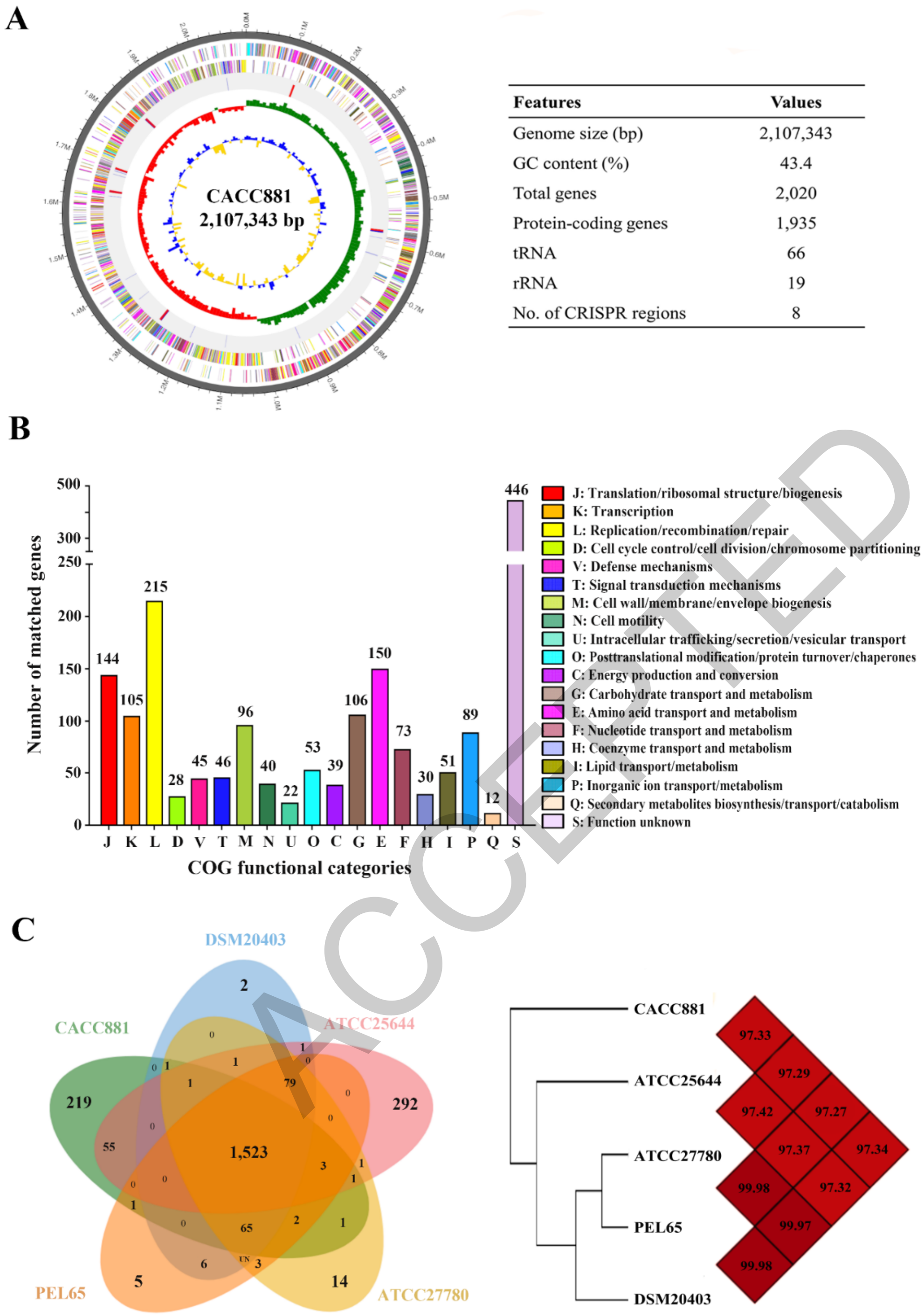
COG, Clusters of Orthologous Groups; POG, pan-genome orthologous group; J: translation, ribosomal structure, and biogenesis; K: transcription; L: replication, recombination, and repair; D: cell cycle control, cell division, and chromosome partitioning; V: defense mechanisms; T: signal transduction mechanisms; 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

Table 2. Predicted genes involved in the potential probiotic properties of *L. 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

Figure legends



Features	Values
Genome size (bp)	2,107,343
GC content (%)	43.4
Total genes	2,020
Protein-coding genes	1,935
tRNA	66
rRNA	19
No. of CRISPR regions	8

Figure 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.