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Complete genome sequence of *Latilactobacillus curvatus* CACC879 and its functional probiotic properties

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

Abstract

Latilactobacillus curvatus CACC879 originated from swine feces in Korea, and its probiotic properties have been analyzed. The complete genome of strain CACC879 contained one chromosome 1,398,247 bp in length and three circular plasmids, namely, pCACC879-1 (591,981 bp), pCACC879-2 (14,542 base pairs [bp]), and pCACC879-3 (45,393 bp). The complete genome encodes a total of 2,077 genes, including 25 rRNA genes and 90 tRNA genes. In addition, probiotic stability- genes acid/bile related to salts tolerance, the biosynthesis of cobalamin (vitamin B12), riboflavin (vitamin B2), and CRISPR/Cas9 were found in the whole genomes. Remarkably, *L. curvatus* CACC879 contained the antioxidant-related (peroxiredoxin) and bacteriocin-related genes (*lysM* and *blpA*). Overall, these results demonstrate that *L. curvatus* CACC879 is a functional probiotic candidate for animal industry applications.

Keywords: *Latilactobacillus curvatus*, Swine, Probiotics, PacBio, Genome sequence

Lactic acid bacteria, such as *Latilactobacillus*, are useful microbes that produce healthy metabolites, including bacteriocins and organic acids (such as lactic acid), that can regulate the gut microbiome balance [1]. Lactic acid bacteria also confer health benefits via diverse mechanisms, such as acid and bile tolerance, epithelial cell adherence, intestinal barrier buildup, and immune system modulation [2]. *Latilactobacillus curvatus* is a potential probiotic strain that produces various bacteriocins and metabolites and exhibits immunomodulatory activity [3,4]. In this study, the genome of *L. curvatus* CACC879 was sequenced and fully assembled to elucidate the genetic factors associated with its probiotic characteristics.

L. curvatus CACC879 was isolated from swine feces in Korea, and the isolate was cultured in De Man, Rogosa, and Sharpe (MRS) medium for 18 h at 37℃. The genomic DNA of *L. curvatus* CACC879 was extracted and purified using the DNeasy UltraClean kit (Qiagen, Hilden, Germany) and sequenced using the PacBio Sequel (Pacific Biosciences, Menlo Park, CA, USA) sequencing platform. *De novo* assembly was performed using PacBio SMRT analysis software (version 2.3.0; Pacific

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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, Son S.
Software: Park S.
Validation: Park S, Son S, Kim DH.
Investigation: Kim Y.
Writing - original draft: Park S.
Writing - review & editing: Park S, Son S, Park MA, 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.

Biosciences) [5]. The EggNOG 5.0 database (http://eggnog5.embl.de) was used to classify all genes into clusters of ortholog gene (COG) / non-supervised orthologous group (NOG) categories. Functional annotations of the predicted coding sequences (CDSs) were compared with the Swiss-Prot and Kyoto Encyclopedia of Genes and Genomes (KEGG) [6]. The genome sequence of CACC879 was compared with other reference strains by Orthologous average nucleotide identity (OrthoANI; http://www.ezbiocloud.net/tools/orthoani) [7].

The whole genome of strain CACC879 consisted of one circular chromosome 1,398,247 bp in length (41.9% guanine-cytosine [GC]) along with three plasmids designated pCACC879-1 (591,981 bp, 42.2% GC), pCACC879-2 (14,542 bp, 45.2% GC), and pCACC879-3 (45,393 bp, 41.2% GC) (Table 1 and Fig. 1A). The genome of strain CACC879 contains 2,077 CDSs and 115 non-coding genes (25 rRNA and 90 tRNA genes) (Table 1). In addition, a total of 1,874 proteins (90.2%) were matched and classified into 19 COG functional categories (Fig. 1B). The most abundant COG categories were associated with replication, recombination, and repair (12.7%); translation, ribosomal structure, and biogenesis (7.8%); transcription (7.6%), carbohydrate transport, and metabolism (7.5%); and cell wall/membrane/envelope biogenesis (5.7%), excluding those with unknown function (29.7%). Compared with the genome sequence of reference strains, the genome of strain CACC879 was the most similar to that of the reference strains *L. curvatus* DSM 20019 (99.4%) and Wikim38 (99.0%) (Fig. 1C). The CACC879 strain showed common probiotic properties including the CRISPR-associated endonuclease (Cas9) for antiviral-related mechanisms and the biosynthesis of vitamin B groups (*ribF* and *pduO*), bacteriocin (*lysM*), and antioxidant (*tpx*), compared to the reference strains [8-10]. Additionally, we confirmed that strain CACC879 harbors genes associated with common probiotic properties, including acid tolerance (*clpB* and *grpE*), lactate synthesis (*ldh* and L-lactate dehydrogenase), and cell adhesion (*sotA*) (Table 2). Interestingly, the CACC879 genome contained the *dltB* and *dltD* genes associated with the modulation of the host immune response, but the reference strains did not. These findings will serve as a reference for further studies on *L. curvatus* and provide a scientific basis for functional probiotic development.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER(S)

The whole-genome sequence of *L. curvatus* strain CACC879 (KACC 92511) has been deposited in GenBank under accession numbers CP117683 (chromosome) and CP117684 to CP117686 (plasmids). The BioProject and BioSample accession numbers are PRJNA932593 and SAMN33197937.

Table 1. Genome features of *Latilactobacillus curvatus* CACC879

Properties	Chromosome	Plasmids			Total
	CACC879	pCACC879-1	pCACC879-2	pCACC879-3	
Length (bp)	1,398,247	591,981	14,542	45,393	2,050,163
GC content (%)	41.9	42.2	45.2	41.2	42.0
CDSs	1,421	596	11	49	2,077
tRNA	35	27	28	–	90
rRNA	14	8	3	–	25
CRISPR regions	1	–	–	–	1

bp, base pair; GC, guanine and cytosine; CDSs, coding DNA sequences; tRNA, transfer RNA; rRNA, ribosomal RNA; CRISPR, clustered regularly interspaced short palindromic repeats.

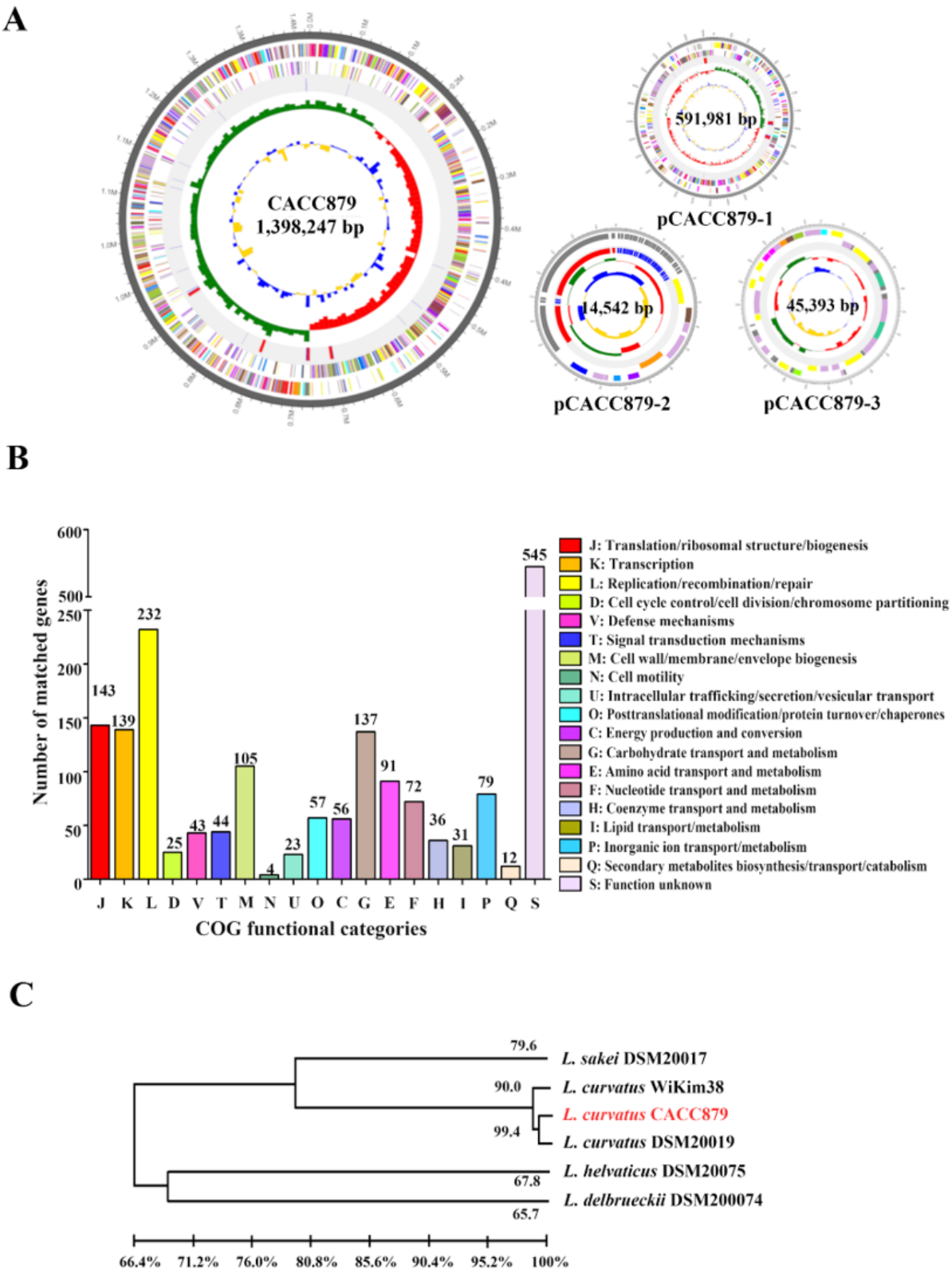

Fig. 1. Genome features of *Latilactobacillus curvatus* CACC879. (A) Circular genome mapping of *L. curvatus* CACC879. (B) Functional classification of clusters of orthologous groups (COG). (C) Orthologous average nucleotide identity (OrthoANI) values of *L. curvatus* CACC879 compared to other reference strains.

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Table 2. Predicted CDSs involved in *Latilactobacillus curvatus* CACC879 probiotic potency

Predicted function	Gene	Start	End	Length (bp)
CRISPR-associated endonuclease	Cas9	31,830	35,723	3,894
Antimicrobial activity-related	*lysM*	1,089,047	1,090,990	1,944
Bacteriocin (Class II)-related	*blpA*	c557,488	c558,636	1,149
Lactate synthesis	*ldh*	c903,407	c904,384	978
Acid tolerance				
Chaperone protein ClpB	*clpB*	706,508	707,140	633
Chaperone protein GrpE	*grpE*	1,335,554	1,336,165	612
ClC family H(+)/Cl(−) exchange transporter	*eriC*	1,148,397	1,149,971	1,575
Sodium hydrogen exchanger family protein	*nhaP*	1,219,349	1,221,493	2145
F0F1 ATP synthase subunit A	*atpB*	17,075	17,788	714
F0F1 ATP synthase subunit B	*atpF*	18,084	18,605	522
F0F1 ATP synthase subunit C	*atpE*	17,807	18,019	213
F0F1 ATP synthase subunit delta	*atpH*	18,592	19,134	543
Bile salts tolerance	*cbh*	c246,195	C246,716	522
Cell adhesion	*sotA*	73,428	74,090	663
Stress response or protection				
Chaperone protein DnaK	*dnaK*	1,336,202	1,338,037	1,836
Chaperone protein DnaJ	*dnaJ*	1,338,161	1,339,309	1,149
Triose-phosphate isomerase	*tpiA*	c532,378	c533,133	756
Biosynthesis of vitamin B groups				
Riboflavin (B2)	*ribF*	1,332,094	1,333,047	954
	ribT	c271,721	c272,089	369
Cobalamin (B12)	*pduO*	58,751	59,305	555
Modulation of Immune response				
D-alanyl-lipoteichoic acid biosynthesis proteins	*dltB*	c220,566	c221,774	1,209
	dltD	c219,030	c220,298	1,269
Antioxidant (peroxiredoxin)	*tpx*	565,480	565,974	495
	tpxA	780,278	780,601	324

CDSs, coding sequences; bp, base pair; CRISPR, clustered regularly interspaced short palindromic repeats.

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