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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Complete genome sequence of *Pediococcus acidilactici* CACC 537 isolated from canine
Running Title (within 10 words)	Genomic feature of *Pediococcus acidilactici*
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Authors' contributions Please specify the authors' role using this form.	Conceptualization: Kim Y Data curation: Kim J.-A., Jang H-J. Formal analysis: Kim J.-A. Methodology: Kim J.-A., Son YK Software: Kim J.-A. Validation: Kim J.-A., Kim D.-H., Investigation: Kim Y Writing - original draft: Kim Y, Writing - review & editing: Kim Y,
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Abstract

Pedi coccus acidilactici CACC 537 was isolated from canine feces and reported to have probiotic properties. We aimed to characterize the potential probiotic properties of this strain by functional genomic analysis. Complete genome sequencing of *P. acidilactici* CACC 537 was performed using a PacBio RSII and Illumina platform, and contained one circular chromosome (2.0 Mb) with a 42% G + C content. The sequences were annotation revealed 1,897 protein-coding sequences, 15 rRNAs, and 56 tRNAs. It was determined that *P. acidilactici* CACC 537 genome carries genes known to be involved in the immune system, defense mechanisms, restriction-modification (R-M), and the CRISPR system. CACC 537 was shown to be beneficial in preventing pathogen infection during the fermentation process, help host immunity, and maintain intestinal health. These results provide for a comprehensive understanding of *P. acidilactici* and the development of industrial probiotic feed additives that can help improve host immunity and intestinal health.

Keywords: *Pediococcus acidilactici*, Canine, Whole-genome sequencing

Lactic acid bacteria (LAB) are mostly used as probiotics in functional foods and feed additives [1]. Among them, *Pediococcus* sp. is used as a beneficial microorganism in the context of food and livestock microbiology, and it has been reported that *P. acidilactici* CACC 537 (KACC 8198BP) has acid and bile tolerance, intestinal adhesion activity, and antibacterial activity against livestock pathogens [2].

This study attempted to genetically determine the useful effects and functions of CACC537 using whole-genome sequencing. The complete genome of CACC 537 was sequenced using a Pacific Biosciences (PacBio, USA) RS II and Illumina (USA) platform with a SMRTbell^{TM} template at Macrogen Inc. (Seoul, Korea), and the reads were assembled of the using HGAP version 3.0 [3]. The annotation of the sequence used automatic results from the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) and analyzing Rapid Annotations using Subsystems Technology (RAST) tools [4]. The complete genome of CACC537 consists of one circular chromosome with a total length of 2,035,984 bp and a DNA G + C content of 42.0%. it includes 1,897 protein-coding genes (CDSs) and 71 RNA genes (15 rRNA and 56 tRNA genes) (Table 1 and Fig. 1A). The 1,782 genes were categorized as functional proteins based on designations through the Clusters of Orthologous Groups (COGs) database. Most assigned CDSs were found to be involved in carbohydrate transport and metabolism; transcription; translation, ribosomal structure and biogenesis; replication, recombination and repair; and cell wall/membrane/envelope biogenesis (225, 146, 142, 107, and 104 genes, respectively) (Fig. 1B).

In the genome of strain CACC 537, the presence of *ldhD*, *ldhL*, *pyk*, *eno*, *fbaA*, *pfk*, and *pgi*, which are involved in lactic acid production as key genes of homo-fermentation in the Embden-Meyerhof-Parnas (EMP) pathway, was detected [5]. We also identified genes encoding lysozymes, chitinases, and proteases, commonly predicted as able to exert antibacterial functions against pathogens; and the *fab* gene cluster, in charge of producing fatty acids that can protect the intestinal mucosal from pathogens. Interestingly, the short-chain fatty acids (SCFA) produced as metabolic by-products play an important role in intestinal homeostasis and have been reported to be associated with immune enhancement and liver function improvement [6-8]. In addition, we identified a type II-A CRISPR/CRISPR-related (Cas) gene with two CRISPR (1 and 2) regions, and a CRISPR 9 region; and genes involved in immune and defense mechanisms, including some for restriction-modification (R-M) systems. This strain prevents fermentation failure due to bacterial phage and pathogen infection in the fermentation process, and has an intestinal function to help host immunity and maintain intestinal health [9-10] (Table 2).

This study on the complete genome sequence of *P. acidilactici* CACC 537 showed that it has potential probiotic effects, and thus may be useful for the development of health-promoting products.

Nucleotide sequence accession number

The complete genome of *P. acidilactici* CACC 537 has been deposited in the NCBI GenBank database under the accession number CP048019.

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Tables and Figures

Table 1. Genome overview of *Pediococcus acidilactici* CACC 537

Feature	**Values**
Genome size (bp)	2,035,984
No. of contigs	1
GC content	42.0 %
Protein-coding genes (CDSs)	1,897
rRNA	15
tRNA	56
Genbank Acession No.	CP048019

Table 2. Predicted genes involved in probiotic potency in *Pediococcus acidilactici* CACC537

Predicted function	*Pediococcus acidilactici* **CACC 537**			
	Predicted genes	**Products**	**Gene position**	**Length (bp)**
Homolactic-Fermentation related	*ldhD*	D-lactate dehydrolase	195437..196432	996
	ldhL	L-lactate dehydrolase	940686..941657	972
			c(1521565..1522485)	921
	pyk	Pyruvate kinase	c(408957..410720)	1,764
	eno	Phosphopyruvate hydratase	c(1111207..1112493)	1,287
			1802875..1804197	1,323
	fbaA	Fructose-bisphosphate aldolase	730570..731433	864
	pfk	6-phosphofructokinase	c(410797..411765)	969
			c(552661..553572)	912
	pgi	Glucose-6-phosphate isomerase	c(727794..729146)	1,353
Antimicrobial resistance-related				
Bacteriolytic enzyme	-	Lysozyme	c(465359..466543)	1,185
	-	Chitinase	1707522..1708034	513
			2026125..2027144	1,020
	glup	Rhomboid Protease	125912..126601	690
Fatty acid biosynthesis (Clusters)	*fabI*	Enoyl-(acyl-carrier-protein) reductase	187972..188730	759
	accA,D,C	Acetyl-CoA carboxylase	184990..187955	2,966
	fabZ1	3-hydroxyacyl-(acyl-carrier-protein) dehydratase	184563..184973	411
	accB	Acetyl-CoA carboxylase	184125..184550	426
	fabF,G,D	β-ketoacyl-ACP synthase II, β-ketoacyl-ACP reductase, acyl-carrier protein	181121..184120	3,000
	acpP	Acyl carrier protein	180858..181094	237
	fabH,Z2	β-ketoacyl-ACP synthase III, β-hydroxyacyl-ACP dehydratase	179410..180831	1,422
Restriction-Modification system	*hsdR*	Type I site-specific deoxyribonuclease	1445338..1448211	2,874
	hsdM	Site-specific DNA-methyltransferase	1448232..1449824	1,593
	hsdS	Type I site-specific deoxyribonuclease	1449821..1453105	3,284
Immune system	*cas9*	CRISPR-associated endonuclease	1747125..1751225	4,101
	cas1	CRISPR-associated endonuclease	1751443..1752348	906
	cas2	CRISPR-associated endoribonuclease	1752326..1752631	306
	yajC	Immunogenic membrane protein	c(647443..647826)	384

	cfa	Cyclopropane-fatty-acyl-phospholipid synthase	1113749..1114924	1,176
	dacA	Diadenylate cyclase	1814096..1814947	852
	myh6	TNF receptor protein	1730618..1733290	2,673

c; complement

Figure Legends

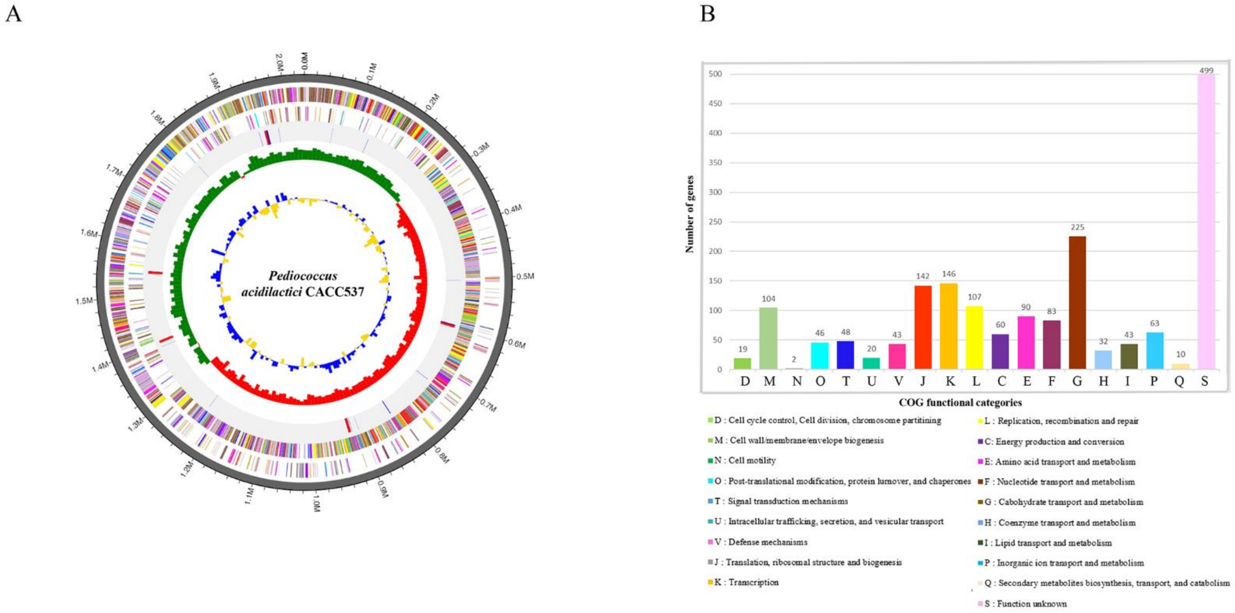

Figure 1. Genome features of *Pediococcus acidilactici* CACC 537. (A) Circular genome maps of the *P. acidilactici* CACC537 chromosome. Circles from the outside to the center denote rRNA and tRNA genes, reverse strand CDSs, forward strand CDSs, GC skew, and GC content. (B) COG functional category annotation numbers on CACC 537 genome.