RESEARCH ARTICLE

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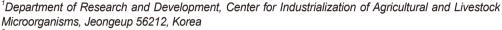


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Complete genome sequence of Pediococcus acidilactici CACC 537 isolated from canine

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Competing interests

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

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 PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA) and Illumina (San Diego, CA, USA) platform with a SMRTbellTM template at Macrogen (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

https://www.ejast.org

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Not applicable.

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: Kim JA, Jang HJ.
Formal analysis: Kim JA.
Methodology: Kim JA, Son YK.
Software: Kim JA.
Validation: Kim JA, Kim DH.
Investigation: Kim Y.
Writing - original draft: Kim Y.
Writing - review & editing: Kim JA, Jang HJ,
Kim DH, Son YK, Kim Y.

Ethics approval and consent to participate

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

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

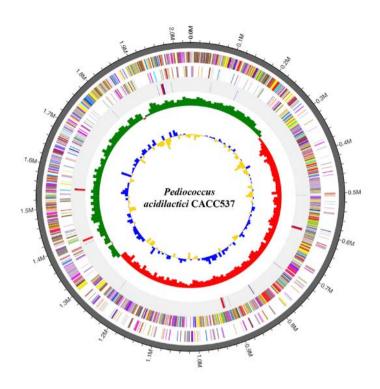
The complete genome of *P. acidilactici* CACC 537 has been deposited in the NCBI GenBank database under the accession number CP048019.

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

bp, base pair.

A



В

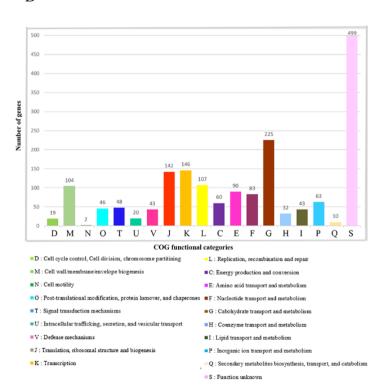


Fig. 1. Genome features of *Pediococcus acidilactici* **CACC 537.** (A) Circular genome maps of the *P. acidilactici* CACC537 chromosome. Circles from the outside to the center denote rRNA and tRNA genes, reverse strand CDSs, forward strand CDSs, GC skew, and GC content. (B) COG functional category annotation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

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

	Pediococcus acidilactici CACC 537				
Predicted function	Predicted genes	Products	Gene position	Length (bp)	
Homolactic-fermentat	ion related				
	ldhD	D-Lactate dehydrolase	195437196432	996	
	ldhL	L-Lactate dehydrolase	940686941657	972	
			c(15215651522485)	921	
	pyk	Pyruvate kinase	c(408957410720)	1,764	
	eno	Phosphopyruvate hydratase	c(11112071112493)	1,287	
			18028751804197	1,323	
	fbaA	Fructose-bisphosphate aldolase	730570731433	864	
	pfk	6-Phosphofructokinase	c(410797411765)	969	
			c(552661553572)	912	
	pgi	Glucose-6-phosphate isomerase	c(727794729146)	1,353	
Antimicrobial resistan	ce-related				
Bacteriolytic enzyme					
	-	Lysozyme	c(465359466543)	1,185	
	-	Chitinase	17075221708034	513	
			20261252027144	1,020	
	glup	Rhomboid protease	125912126601	690	
atty acid biosynthes	is (Clusters)				
	fabl	Enoyl-(acyl-carrier-protein) reductase	187972188730	759	
	accA,D,C	Acetyl-CoA carboxylase	184990187955	2,966	
	fabZ1	3-Hydroxyacyl-(acyl-carrier-protein) dehydratase	184563184973	411	
	accB	Acetyl-CoA carboxylase	184125184550	426	
	fabF,G,D	$\beta\text{-Ketoacyl-ACP}$ synthase II, $\beta\text{-ketoacyl-ACP}$ reductase, acyl-carrier protein	181121184120	3,000	
	acpP	Acyl carrier protein	180858181094	237	
	fabH,Z2	β-Ketoacyl-ACP synthase III, β-hydroxyacyl-ACP dehydratase	179410180831	1,422	
Restriction-modification	on system				
	hsdR	Type I site-specific deoxyribonuclease	14453381448211	2,874	
	hsdM	Site-specific DNA-methyltransferase	14482321449824	1,593	
	hsdS	Type I site-specific deoxyribonuclease	14498211453105	3,284	
mmune system					
	cas9	CRISPR-associated endonuclease	17471251751225	4,101	
	cas1	CRISPR-associated endonuclease	17514431752348	906	
	cas2	CRISPR-associated endoribonuclease	17523261752631	306	
	yajC	Immunogenic membrane protein	c(647443647826)	384	
	cfa	Cyclopropane-fatty-acyl-phospholipid synthase	11137491114924	1,176	
	dacA	Diadenylate cyclase	18140961814947	852	
	myh6	TNF receptor protein	17306181733290	2,673	

bp, base pair; c, complement.

REFERENCES

- Carolina de Oliveira Ribeiro M, Porto de Souza Vandenberghe LP, Spier MR, Paludo KS, Soccol CR, Soccol VT. Evaluation of probiotic properties of Pediococcus acidilactici B14 in association with Lactobacillus acidophilus ATCC 4356 for application in a soy based aerated symbiotic dessert. Braz Arch Biol Technol. 2014;57:755-65. https://doi.org/10.1590/S1516-8913201402258
- Jang HJ, Son S, Kim JA, Jung MY, Choi YJ, Kim DH, et al. Characterization and functional test of canine probiotics. Front Microbiol. 2021;12:625562. https://doi.org/10.3389/fmicb. 2021.625562
- 3. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, et al. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods. 2013;10:563-9. https://doi.org/10.1038/NMETH.2474
- 4. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics. 2008;9:75. https://doi.org/10.1186/1471-2164-9-75
- Peter SB, Qiao Z, Godspower HN, Ajeje SB, Xu M, Zhang X, et al. Biotechnological innovations and therapeutic application of Pediococcus and lactic acid bacteria: the nextgeneration microorganism. Front Bioeng Biotechnol. 2022;9:802031. https://doi.org/10.3389/ fbioe.2021.802031
- Markowiak-Kopeć P, Śliżewska K. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. Nutrients. 2020;12:1107. https://doi.org/10.3390/ nu12041107
- 7. van der Hee B, Wells JM. Microbial regulation of host physiology by short-chain fatty acids. Trends Microbiol. 2021;29:700-12. https://doi.org/10.1016/j.tim.2021.02.001
- 8. Jiang S, Cai L, Lv L, Li L. Pediococcus pentosaceus, a future additive or probiotic candidate. Microb Cell Fact. 2021;20:45. https://doi.org/10.1186/s12934-021-01537-y
- Surachat K, Kantachote D, Deachamag P, Wonglapsuwan M. Genomic insight into Pediococcus acidilactici HN9, a potential probiotic strain isolated from the traditional Thai-style fermented beef nhang. Microorganisms. 2021;9:50. https://doi.org/10.3390/ microorganisms9010050
- Roberts A, Barrangou R. Applications of CRISPR-Cas systems in lactic acid bacteria. FEMS Microbiol Rev. 2020;44:523-37. https://doi.org/10.1093/femsre/fuaa016