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Complete genome sequence of *Lactiplantibacillus plantarum* strain GA_C_14 with potential characteristics applicable in the swine industry

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Abstract

In this study, the complete genome of *Lactiplantibacillus plantarum* strain GA_C_14 was sequenced and analyzed. We isolated the *L. plantarum* strain GA_C_14 from gajami sikhae, the Korean traditional food purchased from a local market in Gangneung, Korea. The genome of the *L. plantarum* strain GA_C_14 consisted of one circular chromosome (3,196,348bp) with a guanine + cytosine (GC) content of 44.7% and one circular plasmid (40,211bp) with a GC content of 38.9%. The genome annotation revealed 3,083 protein-coding sequences (CDSs), 67 tRNAs, as well as 16 rRNAs. The *L. plantarum* strain GA_C_14 possesses enzymes involved in vitamin B_6 metabolism, such as *gapB* (EC 1.2.1.12), *SerC* (EC 2.6.1.52), *dxs* (EC 2.2.1.7), *SerA* (EC 1.1.1.95), *PdxK* (EC 2.7.1.35), and *PdxH* (EC 1.4.3.5). Moreover, the *L. plantarum* strain GA_C_14 harbors the beta-galactosidase (EC 3.2.1.23) enzyme required for synthesizing galactooligosaccharides (GOS). These results suggest that *L. plantarum* strain GA_C_14 could be utilized as a functional probiotic in the swine industry.

Keywords: *Lactiplantibacillus plantarum*, Swine, Whole genome sequencing

Lactiplantibacillus plantarum (*L. plantarum*), formally known as *Lactobacillus plantarum*, stands as one of the most frequently used probiotic strains. Among probiotics, *L. plantarum* is renowned for possessing one of the largest genomes. Its ability to withstand gastric transit enables easy colonization within the intestines of humans and various other mammals [1]. Thriving in diverse ecosystems, *L. plantarum* exhibits exceptional probiotic properties and holds promise as a beneficial addition to the livestock industry [2].

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

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Availability of data and material
The complete genome sequences of *Lactiplantibacillus plantarum* strain GA_C_14 was deposited in GeneBank under the accession numbers CP138485.1 and CP138486.1. The BioSample accession number is SAMN338172807, and BioProject accession number is PRJNA1037459.

Authors' contributions
Conceptualization: Ryu S, Doo H, Kim HB, Lee JH.
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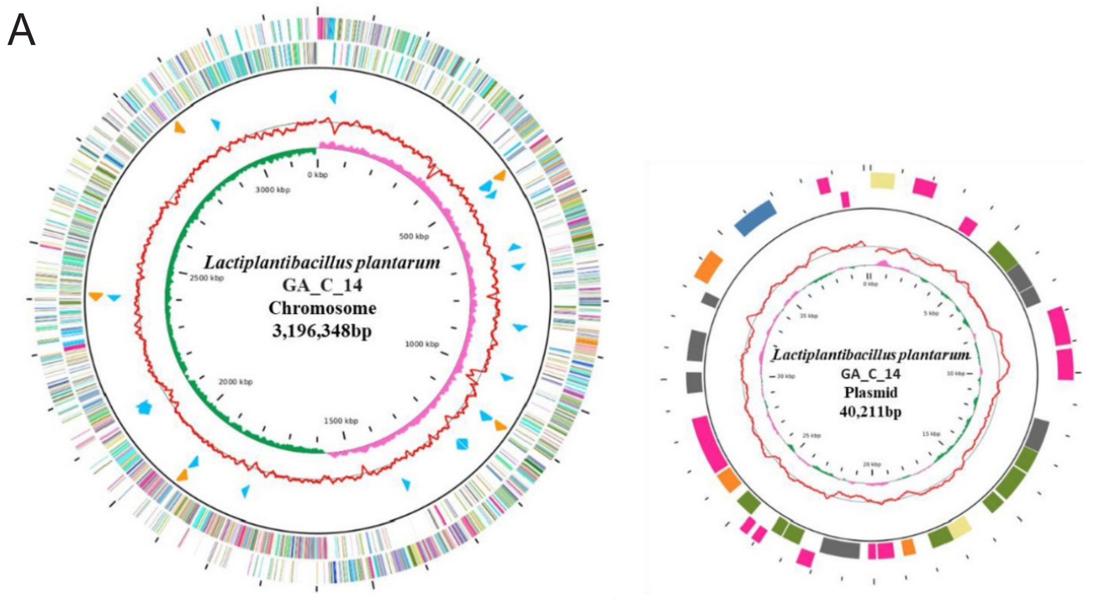
Ethics approval and consent to participate
This article does not require IRB/IACUC approval because there are no human and animal participants.

In this study, we isolated the *L. plantarum* strain GA_C_14 from gajami sikhae, a traditional Korean food purchased from a local market in Gangneung, South Korea. Subsequently, whole genome sequencing of *L. plantarum* strain GA_C_14.was conducted to understand its genomic characteristics, aiming to explore its potential as a probiotic in the livestock industry. *L. plantarum* strain GA_C_14 was cultured using agar solidified by mixing broth supplemented with L-cysteine and Bacto^{TM} Agar (BD Bioscience, Seoul, Korea) in Reinforced clostridial media (BD Bioscience), followed by anaerobic culture at 37°C for 36 hours. The culture was maintained in 25% glycerol solution at -70°C until further use. DNA extraction from the cultured pellet of *L. plantarum* GA_C_14 was performed using the CTAB method. The Oxford Nanopore Technologies MinION platform at eGnome (Seoul, Korea) was employed to fully sequence the complete genome of the *L. plantarum* strain GA_C_14. Initially, Native barcoding sequencing (SQK_NBD114.96 V14) was utilized for library preparation, following the manufacturer's guidelines from Oxford Nanopore Technologies (Oxford, UK). Subsequently, the prepared library was inserted into the MinION MK1b sequencing device (Oxford Nanopore) utilizing a MinION flow cell (MIN114, R10.4.1, Oxford Nanopore), and then verified through the MinKNOW software. A total of 52,921 long read sequences (575,846,933 base pairs) were generated through the Oxford Nanopore sequencing. The Flye assembler v2.9.2 and Canu assembler v1.8 methods were utilized for the de novo assembly. Subsequently, the assembled genome was further refined by employing the Homopolish polisher v0.4.1. The genome assembly's quality was evaluated employing the Quality Assessment Tool for Genome Assemblies (QUAST) v5.0.2 [3]. Benchmarking Universal Single-Copy Orthologs (BUSCO) v5.4.6 was employed for the quantitative evaluation of genome completeness [4]. The Rapid Annotation using Subsystem Technology (RAST) v2.0 tool was utilized for annotating and predicting protein coding genes, tRNA, and rRNA genes in *L. plantarum* strain GA_C_14 [5]. The Clusters of Orthologous Groups (COGs)-based EggNOG-mapper v2.0 was utilized for the functional classification of all predicted protein coding genes. Additionally, the BLASTn method with reference to the Virulence Factor Database (VFDB) was used to predict the presence of virulence factors in *L. plantarum* strain GA_C_14. Identification of antimicrobial resistance genes was performed using the ResFinder v.4.4.0 [6].

The complete genome of the *L. plantarum* strain GA_C_14 comprises one circular chromosome spanning 3,196,348 base pairs with a guanine + cytosine (GC) content of 44.7%. Additionally, it contains one circular plasmid measuring 40,211 bp, exhibiting a GC content of 38.9%. Within the genome of *L. plantarum* strain GA_C_14, a total of 3,083 predicted protein-coding sequences, 67 tRNA, and 16 rRNA genes were identified in. Table 1, Figs. 1A

Table1. Genome features of *Lactiplantibacillus plantarum* strain GA_C_14

Property	Term	
	Chromosome	Plasmid
Contig length (bp)	3,196,348bp	40,211bp
No. of contig	1 (chromosome)	1 (plasmid)
Guanine + cytosine (G + C) content (%)	44.7%	38.9%
Protein-coding genes	3,083	60
rRNA genes	16	-
tRNA genes	67	-
Genbank Accession No.	CP138486.1	CP138485.1



Chromosome size	GC-content(%)	Protein-coding genes	tRNA	rRNA
3,196,348bp	44.7%	3,083	67	16
40,211bp	38.9%	60	-	-

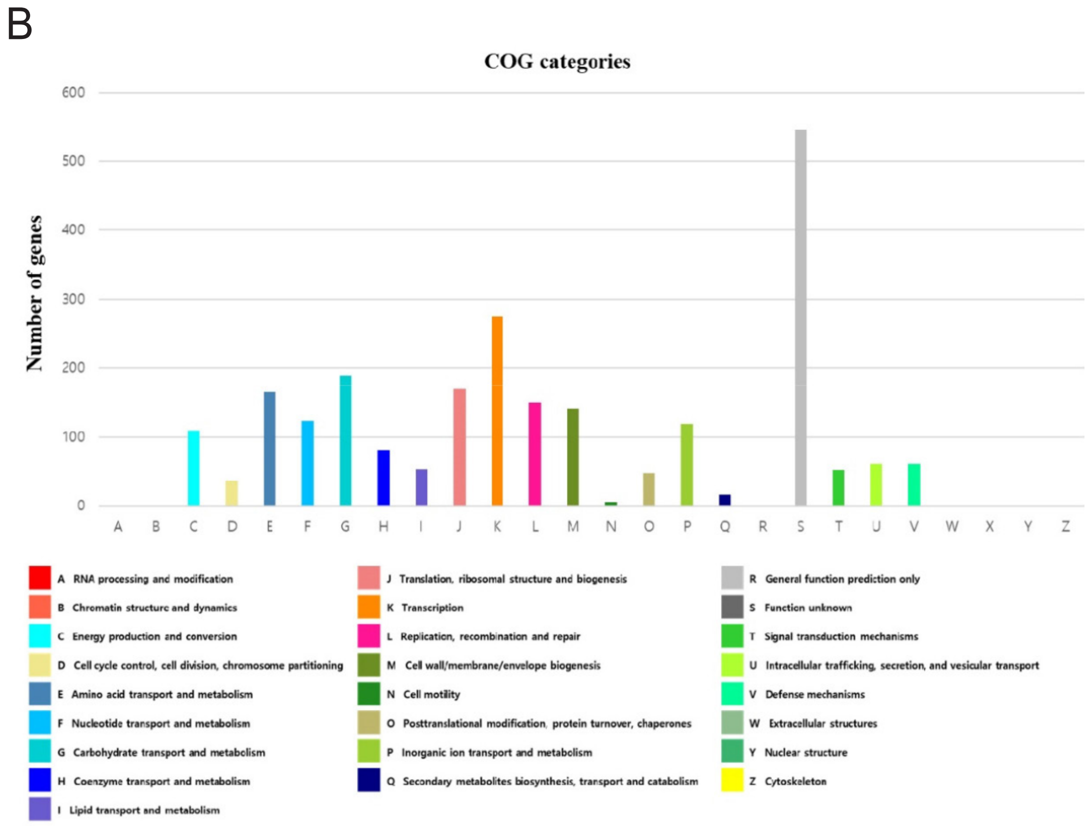


Fig. 1. genome map of *Lactiplantibacillus plantarum* strain GA_C_14 and the functional categorization of predicted protein coding genes. The outer circle illustrates the locations of all annotated gene coding regions (ORFs), while the inner red circle signifies the guanine + cytosine (GC) content. Peaks in pink and green denote GC skew. Meanwhile, rRNA and tRNA operons are indicated by orange and sky-blue arrows, respectively. The color-coded ORFs correspond to their Clusters of Orthologous Groups (COG) assignments in Fig. 1A, and the functional categories of the predicted protein coding genes are depicted in Fig. 1B.

and 1B show the detailed genome features and the map of *L. plantarum* strain GA_C_14.

L. plantarum strain GA_C_14 possesses genes associated with enzymes crucial for effective vitamin B_6 production and usage, as well as for galactooligosaccharides (GOS) synthesis. Specifically, it harbors genes involved in vitamin B_6 metabolism: *gapB* (EC 1.2.1.12), *SerC* (EC 2.6.1.52), *dxs* (EC 2.2.1.7), *SerA* (EC 1.1.1.95), *PdxK* (EC 2.7.1.35) and *PdxH* (EC 1.4.3.5), facilitating the production and utilization of vitamin B_6 [7]. In swine, Vitamin B_6 deficiency can lead to decreased appetite and hindered growth [8]. Additionally, the *L. plantarum* strain GA_C_14 carries the beta-galactosidase (EC 3.2.1.23) enzyme, crucial for synthesizing GOS [9]. This enzyme catalyzes the transgalactosylation reaction in GOS synthesis. Studies have demonstrated that supplementing swine diets with GOS can promote swine growth and enhance intestinal immune status [10]. Therefore, this characteristic suggests that the *L. plantarum* strain GA_C_14 could be used as a potential probiotic candidate for application in the swine industry. Notably, the complete genome of *L. plantarum* strain GA_C_14 does not contain antibiotic resistance genes or virulence factors. In conclusion, our study underscores the potential of *L. plantarum* strain GA_C_14 as a functional probiotic candidate, showing its capacity to positively impact growth outcomes in the swine industry.

References

1. Keum GB, Pandey S, Kim ES, Doo H, Kwak J, Ryu S, et al. Understanding the diversity and roles of the ruminal microbiome. J. Microbiol. 2024;62:217-30. https://doi.org/10.1007/s12275-024-00121-4
2. Yang S, Deng C, Li Y, Li W, Wu Q, Sun Z, et al. Complete genome sequence of Lactiplantibacillus plantarum ST, a potential probiotic strain with antibacterial properties. J Anim Sci Technol. 2022;64:183-6. https://doi.org/10.5187/jast.2022.e7
3. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics. 2013;29:1072-5. https://doi.org/10.1093/bioinformatics/btt086
4. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015;31:3210-2. https://doi.org/10.1093/bioinformatics/btv351
5. 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
6. Chen L, Yang J, Yu J, Ya Z, Sun L, Shen Y, et al. VFDB: a reference database for bacterial virulence factors. Nucleic Acids Res. 2005;33:D325-8. https://doi.org/10.1093/nar/gki008
7. Lee Y, Jaikwang N, Kim S, Jeong J, Sukhoom A, Kim JH, et al. Characterization of a potential probiotic lactiplantibacillus plantarum LRCC5310 by comparative genomic analysis and its vitamin B6 production ability. J Microbiol Biotechnol. 2023;33:644-55. https://doi.org/10.4014/jmb.2211.11016
8. Vijayalakshmy K, Virmani M, Malik R, Rajalakshmi K, Kashturi S. The role of B vitamins in livestock nutrition. J Vet Med Res. 2018;5:1162.
9. Gobinath D, Prapulla SG. Transgalactosylating β-galactosidase from probiotic Lactobacillus plantarum MCC2156: production and permeabilization for use as whole cell biocatalyst. J Food Sci Technol. 2015;52:6003-9. https://doi.org/10.1007/s13197-014-1656-4
10. Tian S, Wang J, Yu H, Wang J, Zhu W. Effects of galacto-oligosaccharides on growth and gut function of newborn suckling piglets. J Anim Sci Biotechnol. 2018;9:75. https://doi.org/10.1186/s40104-018-0290-9