

JAST (Journal of Animal Science and Technology) TITLE PAGE

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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 <i>Lactiplantibacillus plantarum</i> strain GA_C_14 with potential characteristics applicable in the swine industry
Running Title (within 10 words)	Complete genome sequence of <i>Lactiplantibacillus plantarum</i> strain GA_C_14
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Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This research was supported by a grant (22193MFDS538) from Ministry of Food and Drug Safety in 2022
Acknowledgements	Not applicable.
Availability of data and material	The complete genome sequences of <i>Lactiplantibacillus plantarum</i> 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 Please specify the authors' role using this form.	Conceptualization: Ryu S, Doo H, Kim HB, Lee JH Data curation: Kim S, Kim ES, Keum GB Formal analysis: Ryu S, Doo H, Kim ES, Choi Y, Kang J Methodology: Keum GB, Kwak J, Pandey S, Choi Y Validation: Kim S, Kwak J, Kim ES, Kang J

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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.

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

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33 **Abstract (up to 150 words)**

34 In this study, the complete genome of *Lactiplantibacillus plantarum* strain GA_C_14 was sequenced and analyzed.
35 We isolated the *L. plantarum* strain GA_C_14 from gajami sikhae, the Korean traditional food purchased from a
36 local market in Gangneung, South Korea. The genome of the *L. plantarum* strain GA_C_14 consisted of one
37 circular chromosome (3,196,348bp) with a guanine + cytosine (GC) content of 44.7% and one circular plasmid
38 (40,211bp) with a guanine + cytosine (GC) content of 38.9%. The genome annotation revealed 3,083 protein-
39 coding sequences (CDSs), 67 tRNAs, as well as 16 rRNAs. The *L. plantarum* strain GA_C_14 possesses enzymes
40 involved in vitamin B₆ metabolism, such as *gapB* (EC 1.2.1.12), *SerC* (EC 2.6.1.52), *dxs* (EC 2.2.1.7), *SerA* (EC
41 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
42 beta-galactosidase (EC 3.2.1.23) enzyme required for synthesizing galactooligosaccharides (GOS). These results
43 suggest that *L. plantarum* strain GA_C_14 could be utilized as a functional probiotic in the swine industry.

44 **Keywords (3to 6)**

45 *Lactiplantibacillus plantarum*, Swine, Whole genome sequencing

46 **The main text**

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

52 In this study, we isolated the *L. plantarum* strain GA_C_14 from gajami sikhae, a traditional Korean food
53 purchased from a local market in Gangneung, South Korea. Subsequently, whole genome sequencing of *L.*
54 *plantarum* strain GA_C_14 was conducted to understand its genomic characteristics, aiming to explore its
55 potential as a probiotic in the livestock industry. *L. plantarum* strain GA_C_14 was cultured using agar solidified
56 by mixing broth supplemented with L-cysteine and Bacto™ Agar (BD Bioscience, SEOUL, South Korea) in
57 Reinforced clostridial media (BD Bioscience, SEOUL, South Korea), followed by anaerobic culture at 37°C for
58 36 hours. The culture was maintained in 25% glycerol solution at -70 °C until further use. DNA extraction from
59 the cultured pellet of *L. plantarum* GA_C_14 was performed using the CTAB method. The Oxford Nanopore
60 Technologies MinION platform at eGnome (Seoul, South Korea) was employed to fully sequence the complete
61 genome of the *L. plantarum* strain GA_C_14. Initially, Native barcoding sequencing (SQK_NBD114.96 V14)
62 was utilized for library preparation, following the manufacturer's guidelines from Oxford Nanopore Technologies
63 (Oxford, UK). Subsequently, the prepared library was inserted into the MinION MK1b sequencing device (Oxford
64 Nanopore) utilizing a MinION flow cell (MIN114, R10.4.1, Oxford Nanopore), and then verified through the
65 MinKNOW software. A total of 52,921 long read sequences (575,846,933 base pairs) were generated through the
66 Oxford Nanopore sequencing. The Flye assembler v2.9.2 and Canu assembler v1.8 methods were utilized for the
67 de novo assembly. Subsequently, the assembled genome was further refined by employing the Homopolish
68 polisher v0.4.1. The genome assembly's quality was evaluated employing the Quality Assessment Tool for
69 Genome Assemblies (QUAST) v5.0.2 [3]. Benchmarking Universal Single-Copy Orthologs (BUSCO) v5.4.6 was
70 employed for the quantitative evaluation of genome completeness [4]. The Rapid Annotation using Subsystem
71 Technology (RAST) v2.0 tool was utilized for annotating and predicting protein coding genes, tRNA, and rRNA
72 genes in *L. plantarum* strain GA_C_14 [5]. The Clusters of Orthologous Groups (COGs)-based EggNOG-mapper
73 v2.0 was utilized for the functional classification of all predicted protein coding genes. Additionally, the BLASTn
74 method with reference to the Virulence Factor Database (VFDB) was used to predict the presence of virulence

75 factors in *L. plantarum* strain GA_C_14. Identification of antimicrobial resistance genes was performed using the
76 ResFinder v.4.4.0 [6]

77 The complete genome of the *L. plantarum* strain GA_C_14 comprises one circular chromosome spanning
78 3,196,348 base pairs with a guanine + cytosine (GC) content of 44.7%. Additionally, it contains one circular
79 plasmid measuring 40,211 bp, exhibiting a guanine + cytosine (GC) content of 38.9%. Within the genome of *L.*
80 *plantarum* strain GA_C_14, a total of 3,083 predicted protein-coding sequences, 67 tRNA, and 16 rRNA genes
81 were identified in. Table 1, Figure 1A and 1B show the detailed genome features and the map of *L. plantarum*
82 strain GA_C_14.

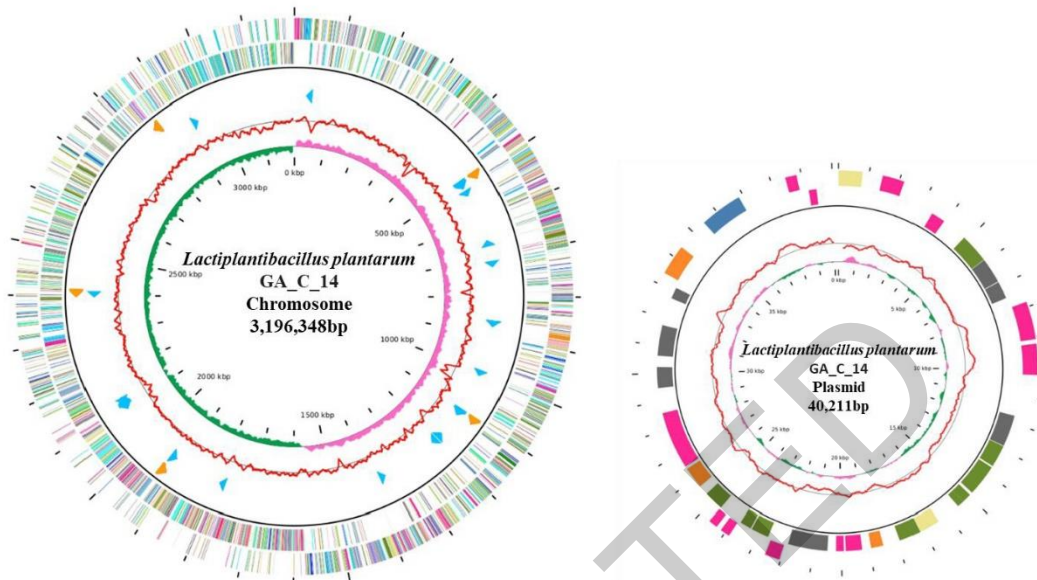
83 *L. plantarum* strain GA_C_14 possesses genes associated with enzymes crucial for effective vitamin B₆
84 production and usage, as well as for galactooligosaccharides (GOS) synthesis. Specifically, it harbors genes
85 involved in vitamin B₆ metabolism: *gapB* (EC 1.2.1.12), *SerC* (EC 2.6.1.52), *dxs* (EC 2.2.1.7), *SerA* (EC 1.1.1.95),
86 *PdxK* (EC 2.7.1.35) and *PdxH* (EC 1.4.3.5), facilitating the production and utilization of vitamin B₆ [7]. In swine,
87 Vitamin B₆ deficiency can lead to decreased appetite and hindered growth [8]. Additionally, the *L. plantarum*
88 strain GA_C_14 carries the beta-galactosidase (EC 3.2.1.23) enzyme, crucial for synthesizing
89 galactooligosaccharides (GOS) [9]. This enzyme catalyzes the transgalactosylation reaction in GOS synthesis.
90 Studies have demonstrated that supplementing swine diets with GOS can promote swine growth and enhance
91 intestinal immune status [10]. Therefore, this characteristic suggests that the *L. plantarum* strain GA_C_14 could
92 be used as a potential probiotic candidate for application in the swine industry. Notably, the complete genome of
93 *L. plantarum* strain GA_C_14 does not contain antibiotic resistance genes or virulence factors. In conclusion, our
94 study underscores the potential of *L. plantarum* strain GA_C_14 as a functional probiotic candidate, showing its
95 capacity to positively impact growth outcomes in the swine industry.

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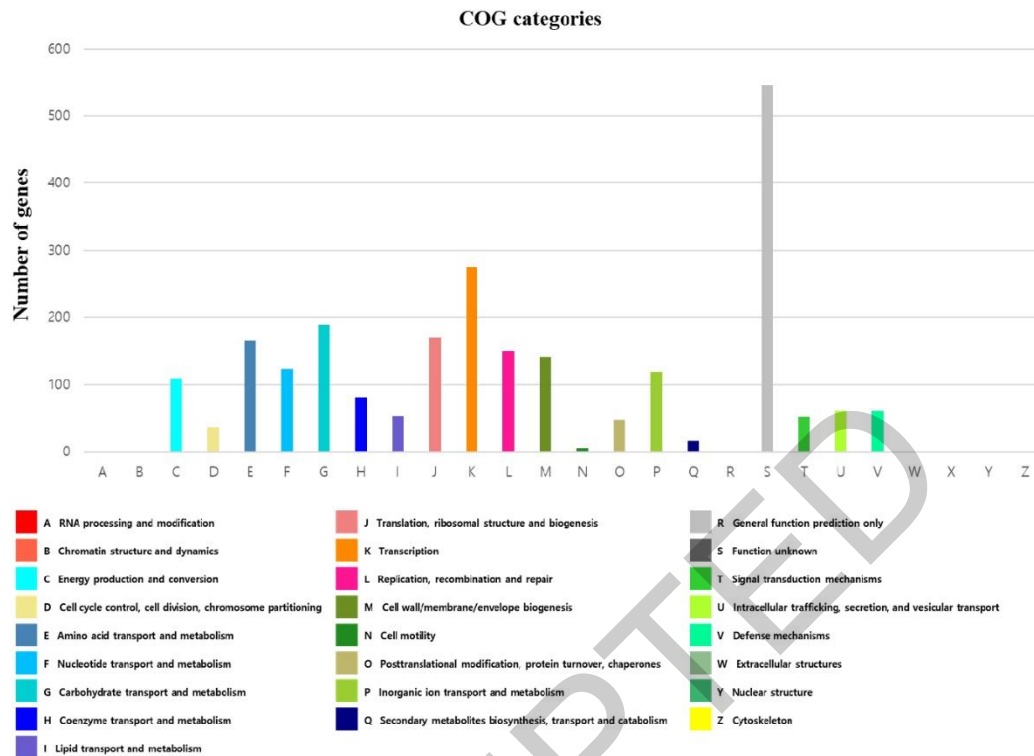
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A

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

B



126

127 **Figure 1. genome map of *L. plantarum* strain GA_C_14 and the functional categorization of predicted**
 128 **protein coding genes.**

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

134

135 **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)	44.7%	38.9%
Protein-coding genes	3,083	60
rRNA genes	16	-
tRNA genes	67	-
Genbank Accession No.	CP138486.1	CP138485.1

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