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Complete genome sequence of candidate probiotic *Limosilactobacillus fermentum* KUFM407

Bogun Kim[1#], Ji yu Heo[2#], Xiaoyue Xu[1], Hyunju Lee[1], Duleepa Pathiraja[1], Jae-Young Kim[2,3], Yi Hyun Choi[2], In-Geol Choi[1]*, Sae Hun Kim[2,3]*

[1]*Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea*
[2]*Department of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea*
[3]*Institute of Life Science and Natural Resources, Korea University, Seoul 02841, Korea*

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#These authors contributed equally to this work.

***Corresponding author**
In-Geol Choi
Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea.
Tel: +82-2-3290-3152
E-mail: igchoi@korea.ac.kr

Sae Hun Kim
Department of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea.
Tel: +82-2-3290-3055
E-mail: saehkim@korea.ac.kr

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ORCID
Bogun Kim
https://orcid.org/0000-0002-5493-1674
Ji yu Heo
https://orcid.org/0009-0009-5334-0125

Abstract

It has been reported that the administration of *Limosilactobacillus fermentum* alleviates diseases such as osteoporosis and colitis. In this study, we report the complete genome sequence of *Limosilactobacillus fermentum* KUFM407, a probiotic strain of LAB isolated from Korean traditional fermented food, Kimchi. Whole genome sequencing of *L. fermentum* KUFM407 was performed on the Illumina MiSeq and Oxford Nanopore MinION platform. The genome consisted of one circular chromosome (2,077,616 base pair [bp]) with a guanine cytosine (GC) content of 51.5% and one circular plasmid sequence (13,931 bp). Genome annotation identified 1,932 protein-coding genes, 15 rRNAs, and 58 tRNAs in the assembly. The function annotation of the predicted proteins revealed genes involved in the biosynthesis of bacteriocin and fatty acids. The complete genome of *L. fermentum* KUFM407 could provide valuable information for the development of new probiotic food and health supplements.

Keywords: *Limosilactobacillus fermentum*, KUFM407, Complete genome sequence, Probiotics

Limosilactobacillus fermentum has been widely used in the fermentation of various foods and is considered a strain with high probiotic potential. Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [1]. Strains of *L. fermentum* have high survival rates in the gastrointestinal tract. They strongly attach to enterocytes and produce antimicrobial compounds. In addition, *L. fermentum* has been shown to benefit host and human health by regulating immune responses and improving intestinal health.

For lactic acid bacteria (LAB) to act as functional probiotic strains, properties such as the ability to adhere to mucosal surfaces and resistance to low pH and high bile concentrations are required [2]. For acid tolerance confirmation, 0.1 mL aliquots of each active culture were inoculated in 10 mL De Man–Rogosa–Sharpe (MRS) broth (BD, Franklin Lakes, NJ, USA) broth acidified to pH 2.5 and supplemented with 1,000 U mL^{-1} of porcine pepsin (Sigma-Aldrich, St. Louis, MO, USA). The samples were then incubated at 37°C for 3 h. To determine bile salt tolerance, 0.1 mL aliquots of each

Xiaoyue Xu
https://orcid.org/0000-0001-9654-8676
Hyunju Lee
https://orcid.org/0009-0000-5423-4812
Duleepa Pathiraja
https://orcid.org/0000-0001-6239-5958
Jae-Young Kim
https://orcid.org/0000-0003-1937-9535
Yi Hyun Choi
https://orcid.org/0009-0006-0513-1503
In-Geol Choi
https://orcid.org/0000-0001-7403-6274
Sae Hun Kim
https://orcid.org/0000-0002-0990-2268

Competing interests
No potential conflict of interest to report.

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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: Choi IG, Kim SH.
Data curation: Kim B, Heo JY, Xu X, Pathiraja D, Choi IG, Kim SH.
Formal analysis: Kim B, Heo JY, Xu X, Pathiraja D.
Methodology: Kim B, Heo JY, Xu X, Pathiraja D.
Software: Kim B, Xu X, Pathiraja D.
Validation: Kim B, Heo JY, Xu X, Lee H, Pathiraja D, Kim JY, Choi YH.
Investigation: Heo JY, Xu X, Lee H, Pathiraja D, Kim JY, Choi YH.
Writing - original draft: Kim B, Heo JY, Kim JY, Choi YH.
Writing - review & editing: Kim B, Heo JY, Xu X, Lee H, Pathiraja D, Kim JY, Choi YH, Choi IG, Kim SH.

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

active culture were inoculated in 10 mL MRS broth containing 0.3% oxgall bile salt (Sigma-Aldrich) and incubated at 37°C for 24h. Following incubation, cell suspensions were spread on MRS agar plates, and viable cell counts were determined through plate counting methods. *L. fermentum* KUFM407 (KUFM407) showed high stability against acid and bile salts (Table 1).

KUFM407 obtained from the Food Microbiology Laboratory, Division of Food Bioscience and Technology, Korea University (Seoul, Korea) was cultivated in MRS broth for 24h at 37°C and sub-cultured three times before the extraction of genomic DNA (gDNA).

Subcultured strains were washed three times with PBS buffer, and 1 mL aliquots of the washed strains were adjusted to the OD600 range of 1.0 to 2.0. Exgene™ Cell SV (Geneall, Seoul, Korea) was used to extract gDNA after gram-positive bacteria-specific pretreatment. The presence of a single strain of KUFM407 was confirmed by gel electrophoresis and 16S rRNA sequencing.

The extracted gDNA was prepared for short-read sequencing using an Illumina® DNA Prep Kit (Illumina, San Diego, CA, USA). Short-read sequencing was performed on an Illumina MiSeq sequencer using the Illumina MiSeq® Reagent Kit v3 (Illumina), resulting in paired-end reads of 300 base pairs (bp) in length. A long-read sequencing library was prepared using an Oxford Nanopore Ligation Sequencing Kit (Oxford Nanopore, Oxford, UK). Long-read sequencing was performed on a MinION sequencing device (Oxford Nanopore) using an R9.4.1 flow cell (Oxford Nanopore). Illumina short-read sequencing yielded 1,699,990 paired-end reads (419,571,925 bp), and Oxford Nanopore long-read sequencing produced 53,365 reads totaling 298,111,808 bp.

The draft genome sequence was constructed from the long reads using Flye assembler (v. 2.9.2) [3] after two polishing iterations. Adapter sequences were removed, and short reads were quality controlled using TrimGalore (v. 0.6.7) [4] in paired-end mode. The quality of the draft genome assembly was improved by error correction with PolyPolish (v. 0.5.0) [5] using quality controlled short reads. Genome and functional annotations of predicted genes were performed using the Prokaryotic Genome Annotation Pipeline (v. 6.4) [6]. Genome completeness was assessed with BUSCO (v. 5.4.6) [7] using the Lactobacillales _odb10 dataset. Default parameters were used for all software unless otherwise noted.

The complete genome sequence of KUFM407 consisted of a circular chromosome (2,077,616 bp) with a guanine + cytosine (G+C) ratio of 51.5% and a circular plasmid sequence of 13,931 bp (Table 2). The genome was 99.7% complete. A total of 2,143 genes, including 1,932 protein-coding,

Table 1. Acid and bile tolerance of *Limosilactobacillus fermentum* KUFM407 (Log CFU/mL)[1)]

Variable	Initial mean counts (0 h)	Resistant to gastric juice (3 h)	Bile tolerance (24 h)
KUFM407	7.71 ± 0.10	7.45 ± 0.06*	8.52 ± 0.08*
Strain A	7.30 ± 0.08	7.24 ± 0.14	4.55 ± 0.04*
L. rhamnosus GG	7.02 ± 0.06	6.97 ± 0.07	8.26 ± 0.23*

[1)]Each value represents mean ± SD from three trials (log CFU/mL).
*$p < 0.05$ (Student's t-test, two tailed).

Table 2. Genome features of *Limosilactobacillus fermentum* KUFM407

	Length (bp)	GC (%)	Depth	CDSs	tRNA	rRNA
Chromosome	2,077,616	51.5	122.0	1,920	58	15
Plasmid	13,931	40.5	36.0	12	0	0
Total	2,091,547	51.4	121.4	1,932	58	15

bp, base pair; G, guanine; C, cytosine; tRNA, transfer RNA; rRNA, ribosomal RNA.

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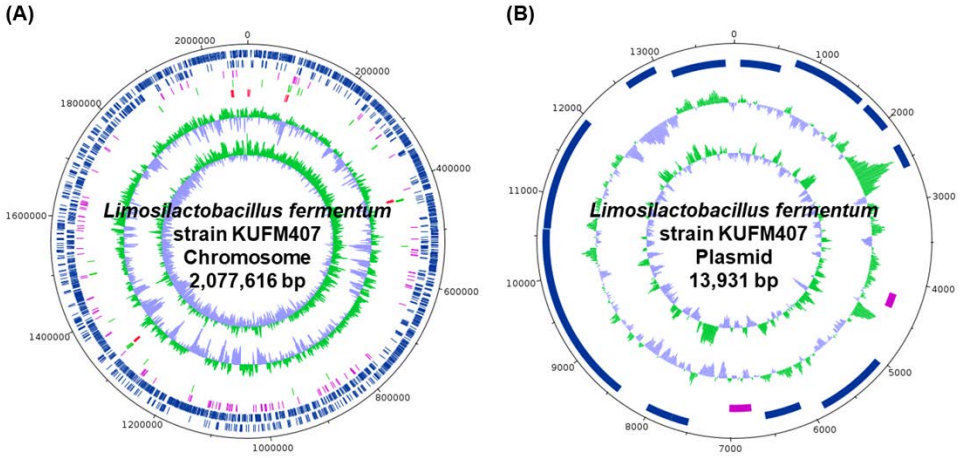

Fig. 1. Circular chromosome and plasmid maps of *Limosilactobacillus fermentum* KUFM407. (A) Chromosome, (B) Plasmid. Marked features are shown from the periphery to the center; protein-coding genes (forward strand), protein-coding genes (reverse strand), pseudogenes, tRNA, rRNA, GC content, and GC skew. bp: base pair; G, guanine; C, cytosine.

Table 3. Genes with biosynthetic functions in *Limosilactobacillus fermentum* KUFM407

Predicted function	Gene name	Functions	Gene position	Length (aa)
Bacteriocin production	*garQ*	Garvicin Q family class II bacteriocin	p(7,514–7,720)	68
	garI	Bacteriocin immunity protein	p(7,720–8,031)	103
	garC	Peptide cleavage/export ABC transporter	p(8,421–10,580)	719
	garD	Bacteriocin secretion accessory protein	p(10,59111,967)	458
Fatty acid biosynthetic gene cluster	*fabZ1*	3-hydroxyacyl-ACP dehydratase	183,265–183,711	148
	marR	MarR family transcriptional regulator	183,796–184,236	146
	fabH	Ketoacyl-ACP synthase III	184,260–185,219	319
	accP	Acyl carrier protein	185,248–185,496	82
	fabD	ACP S-malonyltransferase	185,496–186,443	315
	fabG	3-oxoacyl-ACP reductase FabG	186,427–187,158	243
	fabF	Beta-ketoacyl-ACP synthase II	187,171–188,409	412
	accB	Acetyl-CoA carboxylase biotin carboxyl carrier protein	188,412–188,858	148
	fabZ2	3-hydroxyacyl-ACP dehydratase	188,861–189,295	144
	accC	Acetyl-CoA carboxylase biotin carboxylase subunit	189,316–190,713	465
	accD	Acetyl-CoA carboxylase carboxyltransferase subunit beta	190,682–191,530	282
	accA	Acetyl-CoA carboxylase carboxyltransferase subunit alpha	191,523–192,296	257
	fabI	Enoyl-ACP reductase	192,314–193,078	254

aa, amino acid; p, plasmid.

15 rRNA, and 58 tRNA genes, and 135 pseudogenes were predicted in the genome sequence (Fig. 1). Biological functions were assigned to 1,729 (89.5%) of the protein-coding genes. The most assigned proteins were associated with replication, recombination and repair; amino acid transport and metabolism; translation, ribosomal structure and biogenesis; transcription; and carbohydrate transport and metabolism (207, 170, 155, 137, 123 genes, respectively).

In the plasmid sequence of KUFM407, four genes (*garQ, garI, garC, garD*) known to be involved in the production of the garvicin Q family class II bacteriocin were found [8]. Also, fatty acid biosynthetic gene cluster was identified in the chromosome and short-chain fatty acids such as

acetate, propionate, and butyrate produced by gut microbes are known to have anti-inflammatory effects [9] (Table 3). The genomic information of *L. fermentum* KUFM407 could provide insights for future research on the characteristics of this strain as a functional food and health supplement.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The complete genome sequence has been deposited in NCBI GenBank under accession number GCA_030290995.1. The BioProject accession number is PRJNA981335 and the BioSample accession number is SAMN35673550.

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