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ARTICLE INFORMATION	Fill in information in each box below
<b>Article Type</b>	Genome Announcement
<b>Article Title (within 20 words without abbreviations)</b>	Complete genome sequence of candidate probiotic <i>Limosilactobacillus fermentum</i> KUFM407
<b>Running Title (within 10 words)</b>	Genome of potential probiotic <i>Limosilactobacillus fermentum</i> KUFM407
<b>Author</b>	Bogun Kim <sup>1</sup> , Ji yu Heo <sup>1</sup> , Xiaoyue Xu <sup>1</sup> , Hyunju Lee <sup>1</sup> , Duleepa Pathiraja <sup>1</sup> , Jae-Young Kim <sup>1,2</sup> , Yi Hyun Choi <sup>1</sup> , In-Geol Choi <sup>1</sup> , Sae Hun Kim <sup>1,2</sup>
<b>Affiliation</b>	<sup>1</sup> College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea <sup>2</sup> Institute of Life Science and Natural Resources, Korea University, Seoul 02841, Republic of Korea
<b>ORCID (for more information, please visit <a href="https://orcid.org">https://orcid.org</a>)</b>	Bogun Kim ( <a href="https://orcid.org/0000-0002-5493-1674">https://orcid.org/0000-0002-5493-1674</a> ) Ji yu Heo ( <a href="https://orcid.org/0009-0009-5334-0125">https://orcid.org/0009-0009-5334-0125</a> ) Xiaoyue Xu ( <a href="https://orcid.org/0000-0001-9654-8676">https://orcid.org/0000-0001-9654-8676</a> ) Hyunju Lee ( <a href="https://orcid.org/0009-0000-5423-4812">https://orcid.org/0009-0000-5423-4812</a> ) Duleepa Pathiraja ( <a href="https://orcid.org/0000-0001-6239-5958">https://orcid.org/0000-0001-6239-5958</a> ) Jae-Young Kim ( <a href="https://orcid.org/0000-0003-1937-9535">https://orcid.org/0000-0003-1937-9535</a> ) Yi Hyun Choi ( <a href="https://orcid.org/0009-0006-0513-1503">https://orcid.org/0009-0006-0513-1503</a> ) In-Geol Choi ( <a href="https://orcid.org/0000-0001-7403-6274">https://orcid.org/0000-0001-7403-6274</a> ) Sae Hun Kim ( <a href="https://orcid.org/0000-0002-0990-2268">https://orcid.org/0000-0002-0990-2268</a> )
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3 **CORRESPONDING AUTHOR CONTACT INFORMATION**

<b>For the corresponding author (responsible for correspondence, proofreading, and reprints)</b>	<b>Fill in information in each box below</b>
First name, middle initial, last name	Sae Hun Kim
Email address – this is where your proofs will be sent	saehkim@korea.ac.kr
Secondary Email address	saehkim@gmail.com
Address	College of Life Sciences and Biotechnology, Korea University, Seoul, 02841, Korea
Cell phone number	010-9071-3055
Office phone number	02-3290-3055
Fax number	3290-3499
First name, middle initial, last name	In-Geol Choi
Email address – this is where your proofs will be sent	igchoi@korea.ac.kr
Secondary Email address	igchoi@gmail.com
Address	Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, 02841, Korea
Cell phone number	010-7448-3289
Office phone number	02-3290-3152
Fax number	

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5 **Genome Announcement**

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7 **Complete genome sequence of candidate probiotic *Limosilactobacillus fermentum***

8 **KUFM407**

9 **Title page:** Genome of potential probiotic *Limosilactobacillus fermentum* KUFM407  
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12 Bogun Kim<sup>a</sup>, Ji yu Heo<sup>a</sup>, Xiaoyue Xu<sup>a</sup>, Hyunju Lee<sup>a</sup>, Duleepa Pathiraja<sup>a</sup>, Jae-Young Kim<sup>a, b</sup>, Yi Hyun Choi<sup>a</sup>, In-  
13 Geol Choi<sup>a</sup>, Sae Hun Kim<sup>a, b</sup>

14

15 <sup>a</sup> College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea

16 <sup>b</sup> Institute of Life Science and Natural Resources, Korea University, Seoul 02841, Republic of Korea

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18 **Abstract**

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

29 **Keywords:** *Limosilactobacillus fermentum*; KUFM407; complete genome sequence; probiotics;

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

37 For lactic acid bacteria (LAB) to act as functional probiotic strains, properties such as the ability to adhere to  
 38 mucosal surfaces and resistance to low pH and high bile concentrations are required [2]. For acid tolerance  
 39 confirmation, 0.1 mL aliquots of each active culture were inoculated in 10 mL De Man–Rogosa–Sharpe (MRS)  
 40 broth (BD Co., Franklin Lakes, USA) broth acidified to pH 2.5 and supplemented with 1000 U mL<sup>-1</sup> of porcine  
 41 pepsin (Sigma-Aldrich, St. Louis, MO, USA). The samples were then incubated at 37 °C for 3h. To determine  
 42 bile salt tolerance, 0.1 mL aliquots of each active culture were inoculated in 10 mL MRS broth containing 0.3%  
 43 oxgall bile salt (Sigma-Aldrich, St. Louis, MO, USA) and incubated at 37 °C for 24h. Following incubation, cell  
 44 suspensions were spread on MRS agar plates, and viable cell counts were determined through plate counting  
 45 methods. *L. fermentum* KUFM407 (KUFM407) showed high stability against acid and bile salts (Table 1).

46 Table 1. Acid and bile tolerance of *Limosilactobacillus fermentum* KUFM407 (log CFU/ml)

	Initial mean counts	Resistant to gastric juice (3h)	Bile tolerance (24h)
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*
<i>L. rhamnosus</i> GG	7.02±0.06	6.97±0.07	8.26± 0.23*

47 Each value represents mean ± standard deviation (SD) from three trials (log CFU/ml); Viable counts (log CFU/ml)  
 48 of each strain at 3h, 24h were compared with initial counts (0h); \**p*<0.05 (Student’s t-test, two tailed).

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

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

57 The extracted gDNA was prepared for short-read sequencing using an Illumina® DNA Prep Kit (Illumina, San  
58 Diego, CA, USA). Short-read sequencing was performed on an Illumina MiSeq sequencer using the Illumina  
59 MiSeq® Reagent Kit v3 (Illumina), resulting in paired-end reads of 300 base pairs (bp) in length. A long-read  
60 sequencing library was prepared using an Oxford Nanopore Ligation Sequencing Kit (Oxford Nanopore,  
61 Oxford, UK). Long-read sequencing was performed on a MinION sequencing device (Oxford Nanopore) using  
62 an R9.4.1 flow cell (Oxford Nanopore). Illumina short-read sequencing yielded 1,699,990 paired-end reads  
63 (419,571,925 bp), and Oxford Nanopore long-read sequencing produced 53,365 reads totaling 298,111,808 bp.

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

71 The complete genome sequence of KUFM407 consisted of a circular chromosome (2,077,616 bp) with a G+C  
72 ratio of 51.5% and a circular plasmid sequence of 13,931 bp (Table 2). The genome was 99.7% complete. A total  
73 of 2,143 genes, including 1,932 protein-coding, 15 rRNA, and 58 tRNA genes, and 135 pseudogenes were  
74 predicted in the genome sequence (Fig. 1). Biological functions were assigned to 1,729 (89.5%) of the protein-  
75 coding genes. The most assigned proteins were associated with replication, recombination and repair; amino  
76 acid transport and metabolism; translation, ribosomal structure and biogenesis; transcription; and carbohydrate  
77 transport and metabolism (207, 170, 155, 137, 123 genes, respectively).

78 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

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

80 In the plasmid sequence of KUFM407, four genes (*garQ*, *garI*, *garC*, *garD*) known to be involved in the  
 81 production of the garvicin Q family class II bacteriocin were found [8]. Also, fatty acid biosynthetic gene cluster  
 82 was identified in the chromosome and short-chain fatty acids such as acetate, propionate, and butyrate produced  
 83 by gut microbes are known to have anti-inflammatory effects [9] (Table 3). The genomic information of *L.*  
 84 *fermentum* KUFM407 could provide insights for future research on the characteristics of this strain as a  
 85 functional food and health supplement.

86

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

Predicted function	Gene name	Functions	Gene position	Length (aa)
Bacteriocin production				
	<i>garQ</i>	Garvicin Q family class II bacteriocin	p(7,514 -7,720)	68
	<i>garI</i>	Bacteriocin immunity protein	p(7,720 – 8,031)	103
	<i>garC</i>	Peptide cleavage/export ABC transporter	p(8,421 – 10,580)	719
	<i>garD</i>	Bacteriocin secretion accessory protein	p(10,591 – 11,967)	458
Fatty acid biosynthetic gene cluster				
	<i>fabZ1</i>	3-hydroxyacyl-ACP dehydratase	183,265 - 183,711	148
	<i>marR</i>	MarR family transcriptional regulator	183,796 - 184,236	146
	<i>fabH</i>	Ketoacyl-ACP synthase III	184,260 - 185,219	319
	<i>accP</i>	Acyl carrier protein	185,248 - 185,496	82
	<i>fabD</i>	ACP S-malonyltransferase	185,496 - 186,443	315
	<i>fabG</i>	3-oxoacyl-ACP reductase FabG	186,427 - 187,158	243
	<i>fabF</i>	Beta-ketoacyl-ACP synthase II	187,171 - 188,409	412
	<i>accB</i>	Acetyl-CoA carboxylase biotin carboxyl carrier protein	188,412 - 188,858	148
	<i>fabZ2</i>	3-hydroxyacyl-ACP dehydratase	188,861 - 189,295	144
	<i>accC</i>	Acetyl-CoA carboxylase biotin carboxylase subunit	189,316 - 190,713	465
	<i>accD</i>	Acetyl-CoA carboxylase carboxyltransferase subunit beta	190,682 - 191,530	282
	<i>accA</i>	Acetyl-CoA carboxylase carboxyltransferase subunit alpha	191,523 - 192,296	257
	<i>fabI</i>	Enoyl-ACP reductase	192,314 - 193,078	254

88 aa, amino acid; p, plasmid

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90 **Data Availability**

91 The complete genome sequence has been deposited in NCBI GenBank under accession number  
92 GCA\_030290995.1. The BioProject accession number is PRJNA981335 and the BioSample accession number is  
93 SAMN35673550.

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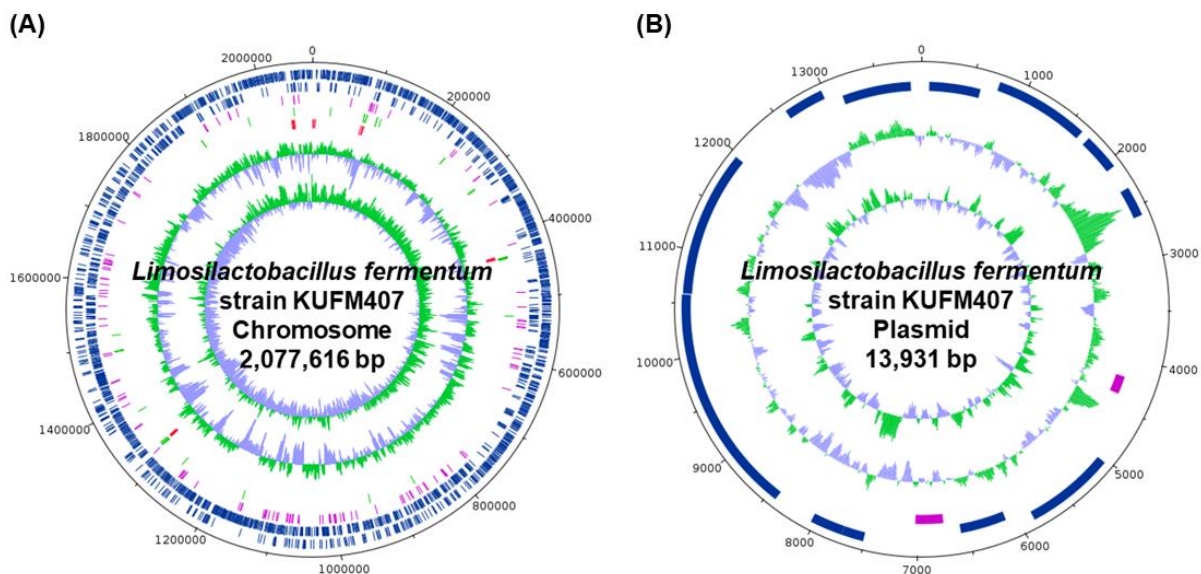
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129 Figure 1



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

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