

Genome Reports

Draft Genome Sequence of *Latilactobacillus sakei* subsp. *sakei* FBL10, a Putative Probiotic Strain Isolated from Saeujeot (salted fermented shrimp)

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Here, we report the draft genome sequence of *Latilactobacillus sakei* subsp. *sakei* FBL10 isolated from Saeujeot (salted fermented shrimp). The draft genome consists of 2,285,672 bp with a G+C content of 41.1% and contains 2,282 coding genes. Genome analysis revealed that clusters associated with bacteriocin production were identified, in addition to several probiotic properties, such as stress resistance factors and aggregation. On the other hand, antibiotic resistance genes and virulence factors were not present. Pangenome analysis for 32 genomes showed 213 unique genes for FBL10 strain. These results demonstrate the beneficial properties of strain FBL10 as a putative probiotic.

Keywords: *Latilactobacillus sakei*, Probiotics, whole-genome sequencing, pangenome analysis

Latilactobacillus sakei is a heterofermentative, rod-shaped Gram-positive lactic acid bacteria. They have been isolated from various niches including vegetables, sourdoughs, meat, and fish. In addition, they play an important role in fermented foods, such as fermented cabbage (kimchi), fermented seafood (jeotgal), and fermented meat products. Indeed, *L. sakei* has been used as a starter culture in fermented sausage and is considered a potential biopreservative of meat products [1]. They also contribute to the safety of the final products due to the production of antimicrobial compounds including sakacin. *L. sakei* has high adaptability to some adverse environmental conditions, which often prevail during preservation, such as high salt concentration, low temperature, low pH, and oxidative stress [2]. Moreover,

they exhibited some health benefits, such as anti-obesity effects and atopic dermatitis [3]. The complete genome sequence was first reported from *L. sakei* 23K which was isolated from French sausage [4]. Subsequently, the genomes of several strains have been sequenced, and genetic diversity and high variability have been revealed [5]. The genome size varied from 1,800 to 2,300 kb, with a variation of approximately 25%. Presently, 34 complete genomes have been sequenced. The purpose of this study was to analyze the draft genome sequence of *L. sakei* subsp. *sakei* FBL10 isolated from Saeujeot to gain deep insight into its probiotic properties.

Saeujeot samples were collected from a local market in Korea. For the isolation of *L. sakei*, 10 g of the sample was homogenized with 90 ml of phosphate-buffered saline. The diluted homogenate was spread on de Man-Rogora-Sharpe (MRS) agar (MBcell, Korea) and incubated at 30°C for 48 h. The bacterial colonies were selected based on colony morphology and then identified

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by Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and 16S rRNA gene sequencing. MALDI-TOF MS was performed using the Microflex LT mass spectrometer (Bruker Daltonics, Germany) with BioTyper database. Genomic DNA was isolated using DNeasy Blood and Tissue kit (Qiagen, Germany) according to the manufacturer's instruction. The library was prepared using Ion Xpress Plus Fragment Library Kit (Thermo Fisher Scientific, USA) and its quality was confirmed using an Agilent 2100 bioanalyzer with the high-sensitivity DNA kit. Draft genome sequencing was performed using an Ion Torrent Personal Genome Machine (Thermo Fisher Scientific) using a 318 semiconductor chip (400 bp

sequencing reads). A total of 5,796,089 reads, with an average read length of 304 bp, were obtained. Reference-based assembly was carried out using SPAdes version 3.1.0 with *L. sakei* 23K (CR936503.1) as the reference genome. Assembly of the reads resulted in 59 contigs > 1,000 bp (1,499 to 615,703 bp; N₅₀ length 138,163 bp). The draft genome size was 2,330,863 bp with a G+C content of 41.1% (Table 1). *L. sakei* subsp. *sakei* FBL10 has a genome size and G+C content similar to *L. sakei* wikim 22, *L. sakei* HUP1, and *L. sakei* WiKim0095. The assembled sequence was annotated by the National Center for Biotechnology Information (NCBI) prokaryotic genome annotation pipeline. The annotation showed that the genome of *L. sakei* subsp. *sakei* FBL10 had 2,282 coding genes.

Table 1. Genome feature of *L. sakei* subsp. *sakei* FBL10.

Feature	Values
Genome size (bp)	2,285,672
G+C content (%)	41.1
Contig N ₅₀	138,163
Contig L ₅₀	4
Number of contigs	59
Number of coding genes	2,282
Number of RNAs	62
Number of subsystems	298

The species prediction of *L. sakei* subsp. *sakei* FBL10 was performed by 16S rRNA gene analysis. The phylogenetic analysis showed that *L. sakei* subsp. *sakei* FBL10 was closest to the *L. sakei* subsp. *sakei* DSM 20017^T (Fig. 1). The 16S rRNA gene similarity showed 99.7 and 99.5% for *L. sakei* subsp. *sakei* DSM 20017^T and *L. sakei* subsp. *carneus* CCUG 31331^T, respectively. Also, the 16S rRNA gene similarity showed 97.7 to 99.2% identities between *L. sakei* subsp. *sakei* FBL10 and other *Latilactobacillus* species (*L. fragifolii* AMBP 162^T, 99.2%; *L. curvatus* NBRC 15884^T, 99.2%; *L.*

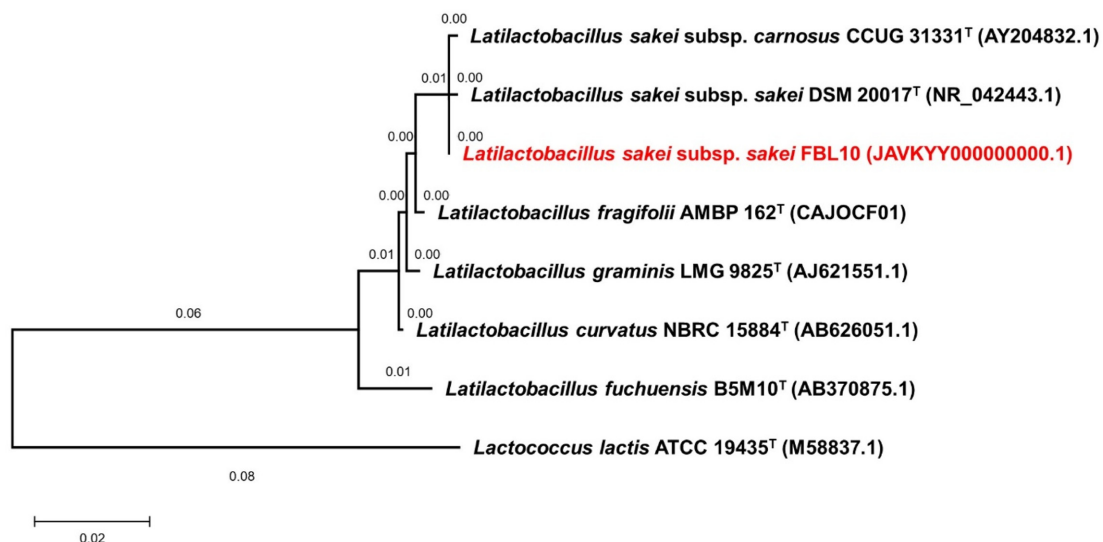


Fig. 1. Phylogenetic tree for *L. sakei* subsp. *sakei* FBL10 strain obtained from this study and other *Latilactobacillus* species based on 16S rRNA gene sequences using the maximum likelihood method. Bootstrap values for 1,000 replicates are displayed at the branching point. The scale bar indicates 0.02 nucleotide substitutes per position. *L. sakei* subsp. *sakei* FBL10 strain obtained from this study is indicated in red letters. The outgroup used *Lactococcus lactis* ATCC 19435^T (M58837.1).

graminis LMG 9825^T, 98.7%; *L. fuchuensis* B5M10^T, 97.7%). The taxonomic position of *L. sakei* subsp. *sakei* FBL10 was confirmed by average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH). ANI and dDDH values were performed using the ANI calculator and Genome-to-Genome Distance Calculator, respectively. The proposed ANI cut-off for the species boundary is $\geq 95\%$, indicating that the strain FBL10 is the same species as *L. sakei* subsp. *sakei* ATCC 15521^T (ANI value, 98.8%) and *L. sakei* subsp. *carneus* DSM 15831^T (ANI value, 97.2%). For the subspecies boundary, the dDDH cut-off value is $> 79\%$. dDDH value showed that the strain FBL10 was *L. sakei* subsp. *sakei* ATCC 15521^T (dDDH value, 91.5%) rather than *L. sakei* subsp. *carneus* DSM 15831^T (dDDH value, 75.3%).

The probiotic-related genes in the *L. sakei* subsp. *sakei* FBL10 were identified by Rapid Annotations using Subsystem Technology version 2.0. The secondary metabolite biosynthesis gene cluster was detected using antiSMASH version 6.0. The genome of *L. sakei* subsp. *sakei* FBL10 contained putative genes involved in acid and bile tolerance, heat or oxidative stress tolerance, and adhesion to intestinal epithelial cells. Genes encoding F₀F₁ ATP synthase (Locus tag: MDR7924316.1 to MDR7924323.1) and chologlycine hydrolase (MDR7924149.1), which are associated with low pH and bile salt tolerance, were identified, thus posing the possibility of its survival in the gastrointestinal tract [6]. Genes encoding chaperone proteins, such as *dnaK* (MDR7924671.1), *dnaJ* (MDR7924672.1), *groES* (MDR7924603.1), and *grpE* (MDR7924670.1) were identified, which are beneficial in tolerating temperature stress [7]. Also, genes associated with the oxidative stress resistance were identified, including *katE* (MDR7924811.1), *trxA* (MDR7924119.1), and *poxL*

(MDR7924701.1). The ability to adhere epithelial cells is critical for probiotic bacteria to persist and thrive [7]. *L. sakei* subsp. *sakei* FBL10 possesses genes encoding cell surface proteins, including *tuf* (MDR7923682.1), *srtA* (MDR7925201.1), and *tpiA* (MDR7923228.1). Cell surface proteins are known to promote bacterial adhesion to epithelial cells [8]. In addition, *L. sakei* subsp. *sakei* FBL10 has gene clusters associated with secondary metabolites production, such as type III polyketide synthase and AgrD-like cyclic lactone autoinducer (Fig. 2). These gene clusters are involved in bacteriocin production [9, 10]. To ensure the safety of *L. sakei* subsp. *sakei* FBL10, antibiotic resistance-associated genes and virulence factor-coding genes were searched for using ResFinder version 4.1 (<https://cge.food.dtu.dk/services/ResFinder/>) and VirulenceFinder version 2.0 (<https://cge.food.dtu.dk/services/VirulenceFinder/>). The result showed that the genome of *L. sakei* subsp. *sakei* FBL10 did not have genes associated with antibiotic resistance and virulence. Pathogenicity analysis by PathogenFinder version 1.1 (<https://cge.food.dtu.dk/services/PathogenFinder/>) showed that this strain was not a pathogen.

For pangenome analysis between the *L. sakei* subsp. *sakei* FBL10 and other *L. sakei* strains, 31 publicly available genomes were downloaded from NCBI (Table 2). The pangenome analysis was confirmed using Bacterial Pan-genome Analysis version 1.3 (<https://icb.res.in/bpga/>). The 32 genomes of *L. sakei* yield 3,672 orthologous genes. Among these, 1,217 genes comprise the core-genome. The accessory-genome and unique-genome contained 1,482 and 973 genes, respectively. The core, accessory, and unique genes were assigned according to their features in the cluster of orthologous groups (COG) databases (<https://www.ncbi.nlm.nih.gov/>

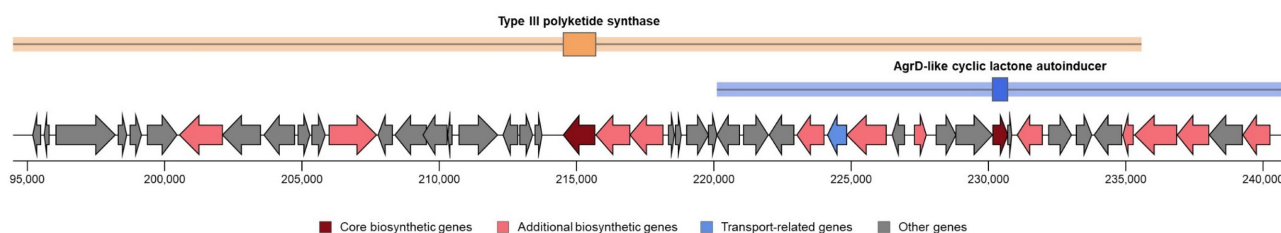


Fig. 2. Genetic loci for type III polyketide synthase and AgrD-like cyclic lactone autoinducer. Arrows represent the direct of gene. The red, pink, blue, and gray arrows indicate core biosynthetic genes, additional biosynthetic genes, transport-related genes, and other genes, respectively.

Table 2. Genomic information used for pangenome analysis.

Organism Name	Strain	Length (bp)	Assembly accession	Assembly level
<i>L. sakei</i> subsp. <i>sakei</i>	23K	1,884,661	GCA_000026065.1	Complete Genome
<i>L. sakei</i>	FAM18311	2,056,563	GCA_002224565.1	Complete Genome
<i>L. sakei</i>	WiKim0063	2,075,520	GCA_002250035.1	Complete Genome
<i>L. sakei</i> subsp. <i>sakei</i>	LT-13	1,943,136	GCA_002370355.1	Complete Genome
<i>L. sakei</i>	LK-145	1,994,264	GCA_002370375.1	Complete Genome
<i>L. sakei</i>	WiKim0072	2,032,521	GCA_003288195.1	Complete Genome
<i>L. sakei</i>	WiKim0074	2,037,873	GCA_003288235.1	Complete Genome
<i>L. sakei</i>	ZFM225	2,015,501	GCA_003627235.1	Complete Genome
<i>L. sakei</i>	ZFM220	2,015,521	GCA_003627275.1	Complete Genome
<i>L. sakei</i>	ZFM229	2,015,489	GCA_003627315.1	Complete Genome
<i>L. sakei</i>	LZ217	2,015,495	GCA_003627875.1	Complete Genome
<i>L. sakei</i>	CBA3614	2,018,071	GCA_009676365.1	Complete Genome
<i>L. sakei</i>	CBA3635	2,062,275	GCA_014081765.1	Complete Genome
<i>L. sakei</i> subsp. <i>sakei</i>	ob4.1	2,030,031	GCA_018437525.1	Complete Genome
<i>L. sakei</i>	TMW 1.46	2,079,718	GCA_023734195.1	Complete Genome
<i>L. sakei</i>	TMW 1.3	1,930,337	GCA_023734215.1	Complete Genome
<i>L. sakei</i> subsp. <i>sakei</i>	TMW 1.1189	1,942,056	GCA_023734235.1	Complete Genome
<i>L. sakei</i>	TMW 1.1239	1,975,742	GCA_023734255.1	Complete Genome
<i>L. sakei</i>	TMW 1.1396	1,935,698	GCA_023734275.1	Complete Genome
<i>L. sakei</i>	TMW 1.1398	2,066,572	GCA_023734295.1	Complete Genome
<i>L. sakei</i>	TMW 1.114	1,976,338	GCA_023734315.1	Complete Genome
<i>L. sakei</i>	TMW 1.417	1,980,463	GCA_023734335.1	Complete Genome
<i>L. sakei</i>	TMW 1.578	1,976,312	GCA_023734355.1	Complete Genome
<i>L. sakei</i>	WiKim0095	2,119,284	GCA_024022895.1	Complete Genome
<i>L. sakei</i>	PMC104	2,015,612	GCA_029536225.1	Complete Genome
<i>L. sakei</i>	HUP1	2,160,337	GCA_029854175.1	Complete Genome
<i>L. sakei</i>	FLEC01	1,957,804	GCA_900234345.1	Complete Genome
<i>L. sakei</i>	J64	2,098,105	GCA_900234355.1	Complete Genome
<i>L. sakei</i>	MFPB16A1401	2,041,677	GCA_900234375.1	Complete Genome
<i>L. sakei</i>	J54	2,011,339	GCA_900234395.1	Complete Genome
<i>L. sakei</i>	MFPB19	2,056,455	GCA_900234405.1	Complete Genome

research/cog). In the core-genome, the group assigned as general function prediction only (R, 13.73%) was the most abundant. The accessory-genome was enriched with genes associated with proteins of carbohydrate transport & metabolism (G, 12.87%) and general function prediction only (R, 12.56%). The unique-genome was enriched with genes encoding proteins of cell wall/membrane/envelope biogenesis (M, 17.54%). Among the 973 unique genes, *L. sakei* subsp. *sakei* FBL10 had 213 unique genes, including genes encoding Clp protease Clp P (MDR7924373.1), type II toxin-antitoxin system

PemK/MazF family toxin (MDR7924721.1), molecular chaperone DnaK (MDR7924671.1), and hypothetical proteins. Our data provide a genetic basis for further research to elucidate the probiotic properties of *L. sakei* subsp. *sakei* FBL10 and promote its consideration as a potential probiotic strain.

Nucleotide Sequence Accession number. The draft genome of *L. sakei* subsp. *sakei* FBL10 has been deposited in GenBank under accession number of JAVKYY000000000.1.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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