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Complete Genome Sequence of *Levilactobacillus brevis* KL251 Isolate from Kimchi

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Abstract

In this study, we performed whole-genome sequencing of *Levilactobacillus brevis* KL251 (KL251) isolated from kimchi. The KL251 genome, characterized by a circular chromosome spanning 2,345,062 base pairs with a GC content of 45.78%, was analyzed. KL251 contains 2,275 coding DNA sequences (CDSs), 56 transfer RNAs (tRNAs), and 4 ribosomal RNAs (rRNAs). Genes associated with gamma-aminobutyric acid (GABA) production and CRISPR-Cas systems were identified and could potentially be used for GABA synthesis and defense against foreign DNA. Additionally, the presence of functional genes involved in isoprenoid biosynthesis, glutathione generation, and redox sensing showed that cellular metabolism and stress responses were important characteristics of this genome. These genomic findings suggest that the KL251 strain could potentially have several applications, including food fermentation, probiotics, dairy product starters, and the development of health-enhancing products.

Keywords

Levilactobacillus brevis KL251, lactic acid bacteria, probiotics, whole genome sequencing

Introduction

Levilactobacillus brevis is a Gram-positive, facultatively anaerobic lactic acid bacteria (LAB) belonging to the family *Lactobacillaceae* within the phylum *Firmicutes*. Formerly classified under the genus *Lactobacillus*, *Levilactobacillus* underwent taxonomic reclassification in 2020 as part of the *Lactobacillus* reorganization based on phylogenomic analysis. This reclassification aimed to resolve phylogenetic inconsistencies and enhance the precision of bacterial taxonomy [1].

L. brevis is known for the hetero-fermentative LAB, converting various carbohydrates into lactic acid, acetic acid, CO₂ or ethanol during fermentation. *L. brevis* is catalase-negative and typically lack cytochrome oxidase activity, traits common among lactic acid bacteria. *L. brevis* is found in various ecological niches, including fermented foods, plant materials, soil, and the gastrointestinal tracts of humans and animals. *L. brevis* is a key lactic acid bacterium found in sauerkraut, pickles, and kimchi, contributing to its unique flavor and health-promoting properties [2,3]. *L. brevis* exhibits probiotic potential, capable of surviving and colonizing the gastrointestinal tract, modulating gut microbiota composition, and conferring health benefits to the host. Their antagonistic activity against pathogenic bacteria, production of antimicrobial substances (e.g., bacteriocins), and immunomodulatory effects further enhance their probiotic properties [4-6].

Genomic studies have provided insights into the genetic diversity, metabolic pathways,



stress response mechanisms, and probiotic attributes of *L. brevis*. Advances in genome sequencing technologies have facilitated comparative genomic analyses, strain-specific trait elucidation, and targeted genetic engineering for improved probiotic and industrial strains.

In this study, we present the complete genome sequence of *L. brevis* KL251, obtained through whole sequencing techniques. Through detailed genomic analysis, we aim to elucidate the genetic basis underlying its fermentative capabilities, probiotic potential, and other relevant physiological traits.

Materials and Methods

Home-made kimchi samples were homogenized in sterile saline and plated on MRS (pH 5.0) agar [7]. Plates were then incubated anaerobically 37°C for 48–72 hours to allow for the growth of white colonies. Biochemical tests such as catalase test, Gram staining, and 16S rRNA gene analysis (Theragen Bio, Korea) were performed to initially identify the isolated strain. The strain identified as *L. brevis*, exhibiting high acid and bile tolerance, was designated as KL251, and its whole genome was analyzed.

The genomic DNA of *L. brevis* KL251 was extracted using the Exgene Cell SV mini kit (GeneAll) according to the instructions of the manufacturer. The complete genome of *L. brevis* KL251 was sequenced at Teragen (Theragen Bio) using the Illumina Novaseq 6000 (Illumina, USA) platform with the TruSeq Nano DNA Sample Prep Kit 150PE (Illumina), which generates short reads. The quality of the assemblies for all genomes were assessed by using QCAST version 4.6.3 (GitHub, USA). Sequence assembly was performed using the following programs: The raw data underwent quality control using fastp (version 0.20.0), the draft genome was assembled with unicycler (version 0.5.0), and errors were corrected using pilon (version 1.24) was utilized [8–10]. The assembled sequences were then assessed using busco (version v5.5.0_cv1). The chromosome contig annotation were performed using rapid prokaryotic genome annotation Prokka (version 1.14.5-2). Using EggNog-mapper (version 2.1.9) [11], we gathered functional annotations for genes or proteins.

Results

The basic genome statistics are provided in Table 1. The complete genome of *L. brevis* KL251 consists of one circular chromosome (2,345,062 bp) with a GC content of 45.78%. According to the genomic results, *L. brevis* KL251 contains 2,275 CDSs, 56 tRNAs, and 4 rRNAs (Fig. 1). Various associated genes have also been identified. It has genes (*gadB_1*, *gadB_2*, *gadC_1*, *gadC_2*, *gadC_3*) involved in GABA production, indicating its potential for GABA synthesis [12]. *L. brevis* KL251 has four CRISPR genes. One of them is associated with the type I-E CRISPR-Cas system, and the second one is associated with the type II CRISPR-Cas system, both of which are potentially active systems. The other two CRISPR gene loci serve as subunits. In addition, functional genes such as Isopentenyl-diphosphate Delta-isomerase, Glutathione amide reductase, and

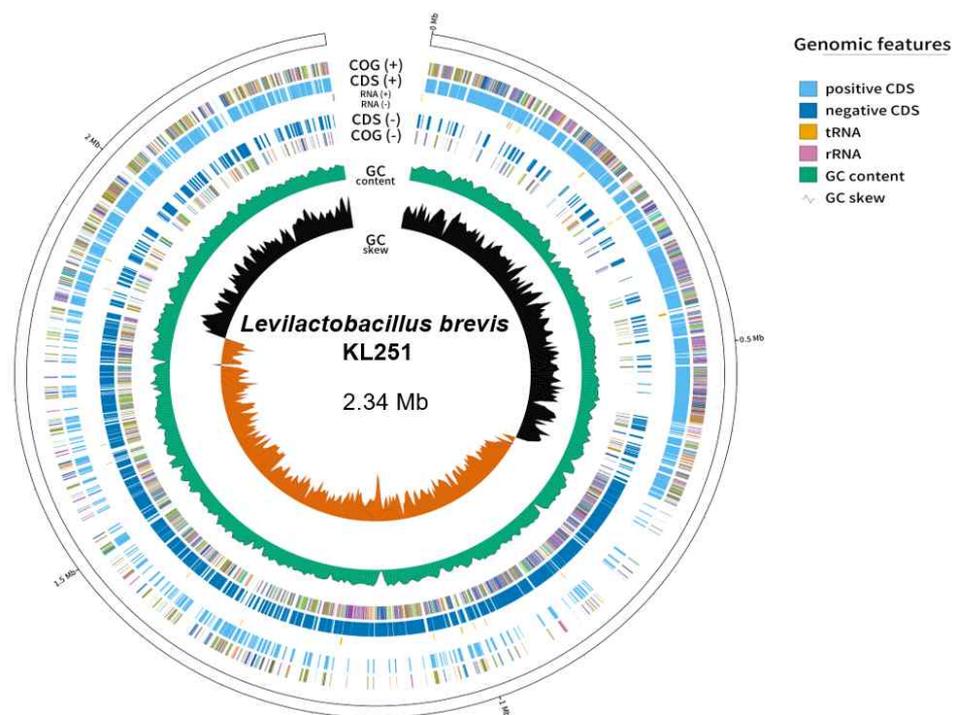


Fig. 1. Circular chromosome maps of *Levilactobacillus brevis* KL251.

Table 1. Genomic features of *Levilactobacillus brevis* KL251

Genomic features	<i>L. brevis</i> KL251 (chromosome)
Genome size (bp)	2,345,062
GC content (%)	45.78
N50 (bp)	2,272,047
rRNA genes	4
tRNA genes	56
tmRNA	1
CDS	2,275

Redox- sensing transcriptional repressor (Rex) are involved in Isoprenoid biosynthesis, the generation of glutathione, and sensing the redox state based on the intracellular NAD⁺/NADH balance, respectively. They play crucial roles in regulating the expression of respiratory genes by detecting the oxidation-reduction (redox) state [13].

These data suggest that KL251, due to its stability and abundance of functional genes, is expected to have a substantial impact on probiotics. The genetic information enhances our understanding of KL251's involvement in fermentation and its potential as a probiotic culture in food and health-related applications.

Conflict of Interest

The authors declare no potential conflict of interest.



Acknowledgements

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