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Whole Genome Sequence Analysis of *Bifidobacterium animalis* subsp. *lactis* KL101 and Comparative Genomics with BB12

Abstract

Bifidobacterium species is a prominent bacterium in the human gut, particularly in infants, where it plays an important role in maintaining gut health. The whole genome sequence of *B. animalis* subsp. *lactis* KL101 (KL101), isolated from infant feces, exhibits a compact structure with a genome size of approximately 1.92 Mbp comprising 1,555 coding sequences. Key chromosomal characteristics are genes encoding bile salt hydrolase and the thioredoxin system, which contribute to bile acid resistance and the oxidative stress response, respectively. Moreover, the genome has a significant number of genes that play a role in carbohydrate metabolism, supporting its probiotic functions. The comparative genomic analysis of the KL101 strain, in comparison to the well-known BB12 strain (*B. animalis* subsp. *lactis* BB12), reveals unique and similar characteristics. Although both strains have a similar GC content, KL101 exhibits unique genomic characteristics that may contribute to its specific adaptations in the infant gut. The results demonstrate that KL101 is highly adapted, with a genome specifically designed to efficiently process carbohydrates, withstand stress, and interact with its host. These findings enhance our understanding of KL101, supporting its potential applications in dietary supplements and health foods aimed at improving gut health.

Keywords: *Bifidobacterium animalis*, KL101, probiotics, whole genome sequencing, BB12

Introduction

Bifidobacterium animalis subsp. *lactis* is a Gram-positive lactic acid bacteria commonly found in the healthy human and animal gut. It is prevalent in the infant gut microbiota, ileum, feces, and mucosa, and also found in the intestines of chickens, rabbits, and the gastrointestinal tracts of pigs and dogs [1,2]. This bacterium survives in the gastrointestinal tract, attaches to human epithelial cells *in vitro*, modulates the fecal microbiota composition, and can prevent gastrointestinal and colonic disorders mediated by microbes [3, 4]. These beneficial effects have established *B. animalis* subsp. *lactis* as a crucial component in the global industry of functional foods, infant formula, and dietary supplements.

The genus *Bifidobacterium* belongs to the order *Bifidobacteriales*, class *Actinobacteria*, and phylum *Actinobacteria*. These non-motile anaerobic bacteria are predominantly isolated from the oral and intestinal tracts of mammals, including humans [5]. Comparative studies have shown that *B. animalis* subsp. *lactis* has a more streamlined genome compared to other species like *Bifidobacterium longum* and *Bifidobacterium breve*, reflecting its specific adaptation to the human gut environment, particularly in infants [6].

This study focuses on the complete genome sequence of *B. animalis* subsp. *lactis* KL101 (KL101), isolated from infant feces, highlighting its structural and functional genomic features. By comparing these features with other *B. animalis* strain such as *B. animalis* subsp. *lactis* BB12 (BB12), we aim to provide a deeper understanding of the genomic adaptations that support its probiotic functions and its potential applications in enhancing gut health.

Additionally, this study contributes to animal industry by exploring how the unique genomic traits of KL101 can be leveraged to improve gut health in livestock. Understanding these traits is crucial for developing probiotics that enhance animal health and productivity, thereby supporting more efficient and sustainable animal husbandry practices.

Materials and Methods

Fecal sample collection and isolation of Bifidobacteria

Fecal samples from six-month-old infant were collected using sterile tools and placed in BL liquid medium overlaid with paraffin oil. The samples were stored at 4°C and processed within 24 hours to preserve bacterial viability. The fecal samples were then diluted with sterile phosphate buffer containing 0.05% L-cysteine (5 mM, pH 7.2) and spread on BL-NPNL agar plates for anaerobic incubation at 37°C for 72 hours using an anaerobic incubator. White colonies that were Gram-positive and catalase-negative were isolated. Finally, 16S rRNA analysis confirmed the identity of *B. animalis*. The identified strain was designated as *B. animalis* subsp *lactis* KL101.

Whole-genome sequencing of *B. animalis* subsp. *lactis* KL101

KL101 was cultured anaerobically in glucose Blood Liver (BL) broth at 37°C for 24 hours. First, genomic DNA extraction was conducted using the Exgene Cell SV mini kit (GeneAll, Seoul, South Korea), following the manufacturer's instructions. Next, the complete genome of KL101 was sequenced at Theragen Bio (Seongnam, South Korea) using the Illumina NovaSeq 6000 platform with the TruSeq Nano DNA Sample Prep Kit 150PE (Illumina Inc., San Diego, CA, USA), generating short reads. Sequence assembly was then performed with fastp (version 0.20.0), Unicycler (version 0.5.0), Pilon (version 1.24), and QUAST (version 5.20). Afterward, the assembled sequences were assessed using BUSCO (version v5.5.0_cv1). Chromosome contig annotation was conducted using Prokka (version 1.14.5-2). Functional annotations for genes and proteins were gathered using eggNOG-mapper (version 2.1.9).

Results and Discussion

Genome Structure and Size

The genome of KL101 consists of a single circular chromosome with a total size of approximately 1,919,804 base pairs (bp) (Table 1). This relatively compact genome is characteristic of the *Bifidobacterium* genus, reflecting a streamlined set of genetic instructions tailored to its ecological niche. The GC (guanine-cytosine) content of the KL101 genome is about 60.4% (Fig. 1), consistent with other bifidobacterial genomes, indicative of its genomic stability and evolutionary adaptations.

When compared to other *Bifidobacterium* species, such as *Bifidobacterium longum* and *Bifidobacterium breve*, and KL101 exhibits a smaller genome size. For instance, the genome of *B. longum* typically spans approximately 2.26 Mb with a GC content of around 60%, while *B. breve* has a genome size of approximately 2.3 Mb [7, 8]. This difference in genome size highlights the evolutionary pressure on KL101 to maintain a streamlined genome that supports its efficient functioning in the infant gut.

Gene Content and Functional Annotation

KL101 contains 1555 coding sequences (CDSs), 53 tRNAs, and 3 rRNAs (Fig. 2). Various associated genes have also been identified. In the genome of KL101, there are genes encoding bile salt hydrolase (EC 3.5.1.24), which may confer resistance to bile acids. Additionally, the genome also contains thioredoxin system-encoding genes, which are alkyl hydroperoxide reductase C (ahpC), putative peroxiredoxin (bcp), thioredoxin reductase (trxB), peptide methionine sulfoxide reductase (MsrAB), divalent metal cation transporter (MntH), and putative thioredoxin 2 (trxC), suggesting potential utilization as antioxidants in the future [8].

Compared to other bifidobacteria, KL101 shows a unique set of genes that enhance its probiotic functionality. For example, the presence of genes related to the oxidative stress response is more pronounced in KL101 compared to *B. longum*, which may contribute to its enhanced survival in the harsh conditions of the gastrointestinal tract [9]. Furthermore, universal CRISPR genes cas1 and cas2, which are crucial for adaptive immunity and genomic stability, have been identified in KL101,.

Carbohydrate Metabolism

A notable feature of the KL101 genome is its extensive array of genes dedicated to carbohydrate metabolism. This includes a variety of glycosyl hydrolases such as β-galactosidases (EC 3.2.1.23) and β-glucosidases (EC 3.2.1.21), which facilitate the degradation of complex carbohydrates into simpler sugars that can be readily absorbed and utilized by the bacterium [10]. The presence of the phosphoketolase pathway (EC 4.1.2.22) is another key element central to the fermentation of pentose and hexose sugars, producing energy and key metabolic intermediates [11].

When compared to other bifidobacteria, such as *B. longum* and *B. breve*, KL101 shows a similar but more specialized profile of carbohydrate-active enzymes. These differences underscore the unique adaptations of KL101 to the infant gut, where milk-derived carbohydrates are a primary nutrient source [12]. For example, *B. longum* has a broader range of carbohydrate metabolism genes, reflecting its adaptation to a more varied adult diet, whereas KL101's genome is finely tuned to efficiently metabolize milk oligosaccharides found in the infant diet [13, 14].

Comparative Genomics with BB12

When comparing the genome of KL101 with that of the well-characterized BB12 strain, several distinct and shared features emerge. BB12 is known for its extensive use in probiotics due to its robust health benefits. In addition, both strains share a high degree of similarity in terms of GC content and general genome organization, indicating a conserved genomic architecture within the subspecies. However, KL101 exhibits unique genomic features that may contribute to its specific adaptations in the infant gut.

Additionally, the genome of KL101 (1.92 Mb) is slightly smaller than that of BB12 (1.96 Mb). This difference, albeit minor, may reflect variations in non-essential genes or strain-specific adaptations. Regarding carbohydrate-active enzymes (CAZymes), KL101 and BB12 both exhibit a rich repertoire, enabling the efficient breakdown of dietary fibers and oligosaccharides. This capability is essential for their role in modulating the gut microbiota and enhancing host health. Moreover, both strains possess genes for bile salt hydrolase and the thioredoxin system, which are crucial for survival in the gastrointestinal tract. However, the exact composition and regulation of these genes may vary, contributing to strain-specific probiotic properties.

The presence of CRISPR-Cas systems in both KL101 and BB12 highlights their ability to defend against phage attacks and maintain genomic stability. The specific array of stress response genes, including those for oxidative stress and heat shock proteins, underscores their resilience in the dynamic gut environment.

Conclusion

The whole genome sequencing and comparative genomic analysis of KL101 provide valuable insights into its probiotic functionalities and adaptations to the infant gut. The unique genomic

traits of KL101 underscore its potential for use in feed supplements and health foods aimed at improving gut health. Further research into these genomic features could facilitate the development of targeted probiotic interventions for both human and animal health.

Sequence Accession Number

The BioProject and BioSample accession numbers for *Bifidobacterium animalis* subsp. *lactis* KL101 are PRJNA1095680 and SAMN40732370.

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198 Table 1. Genomic features of *Bifidobacterium animalis* subsp. *lactis* KL101

Genomic features	*B. animalis* subsp. *lactis* KL101 (Chromosome)
Genome size (bp)	1,919,804
GC content (%)	60.4
N50 (bp)	1,912,929
rRNA genes	3
tRNA genes	53
tmRNA	1
CDS	1,555

199 N50: smallest contig size in which half the genome is represented by contigs of size N50 or
200 larger; rRNA: ribosomal RNA; tRNA: transfer RNA; tmRNA: transfer messenger RNA;
201 CDS: coding sequences

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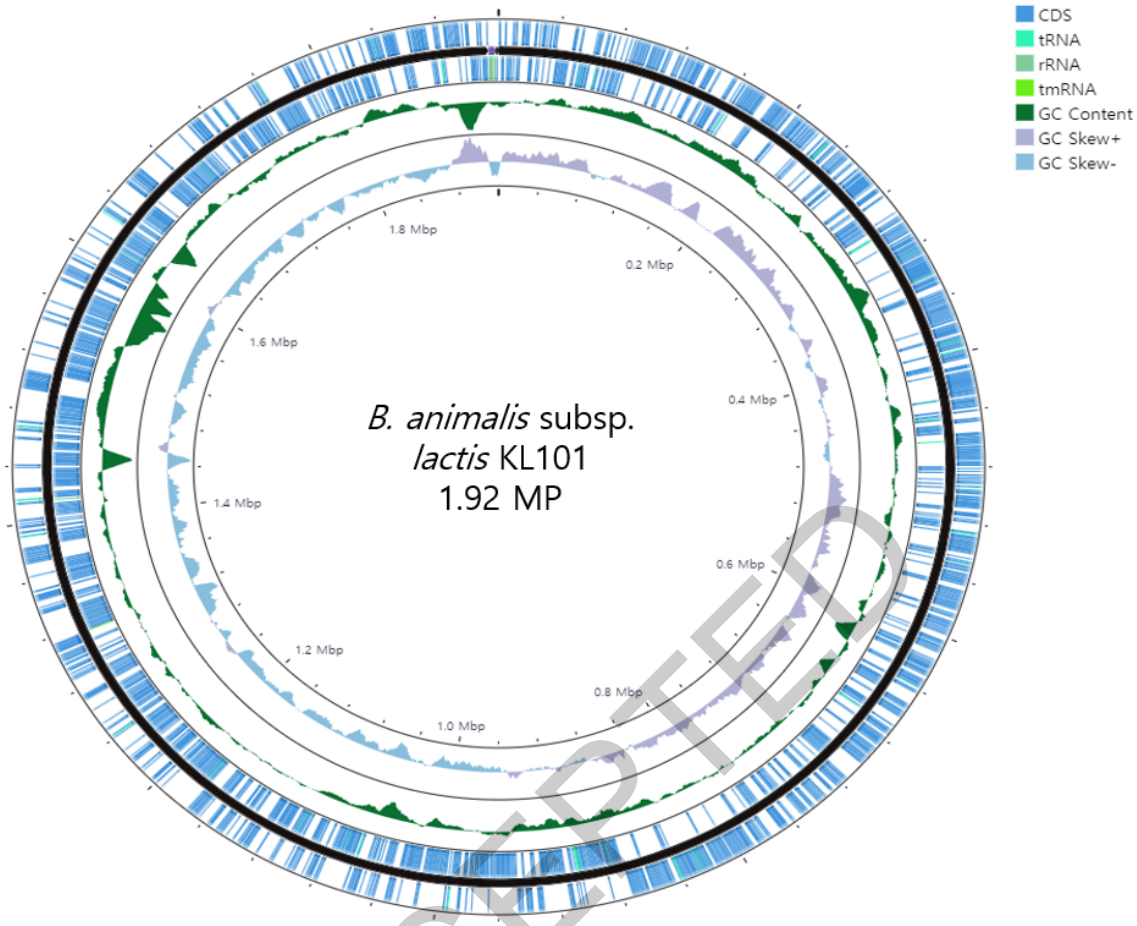

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204 Fig. 1. Circular chromosome contig map of *Bifidobacterium animalis* subsp. *lactis KL101*

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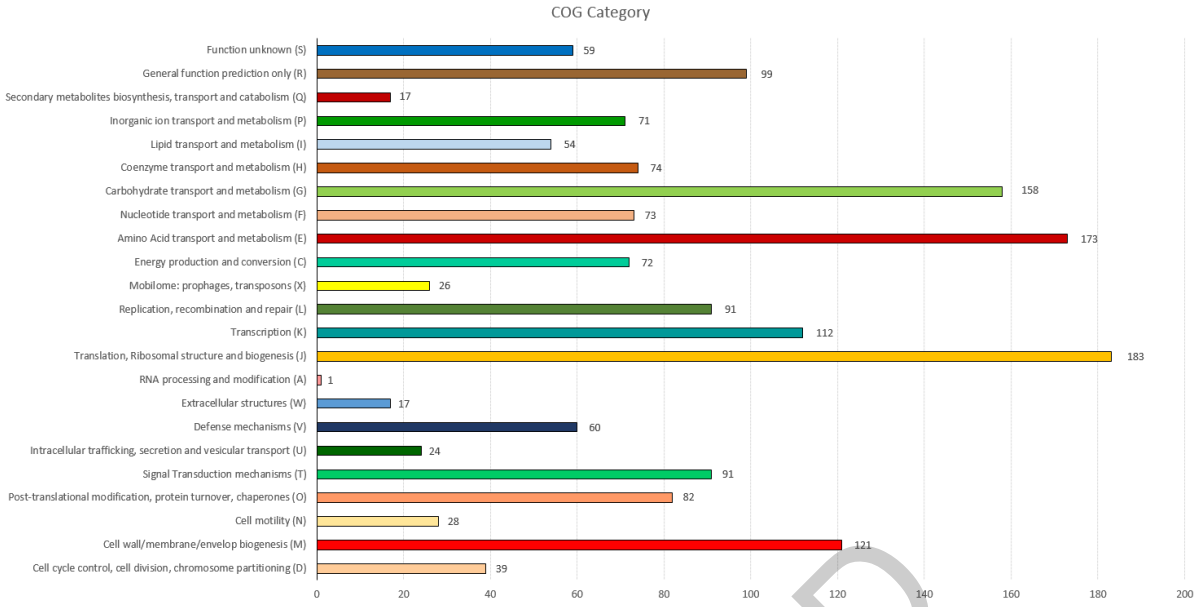

Fig. 2. COG Category of *B. animalis* subsp. *lactis KL101*. Here is a classification of genes: Poorly characterized genes include S and R. Those involved in metabolism are Q, P, I, H, G, F, E, and C. Genes related to information storage and processing are X, L, K, J, and A. Lastly, genes associated with cellular processes and signaling are W, V, U, T, O, N, M, and D.