Complete genome sequence of the *Bifidobacterium animalis* subspecies *lactis* BL3, preventive probiotics for acute colitis and colon cancer

J. Kang1,2, W.-H. Chung1, T.-J. Lim3, S. Lim3 and Y.-D. Nam1,2

1) Research Group of Gut Microbiome, Korea Food Research Institute, Sungnam, 2) Department of Food Biotechnology, Korea University of Science and Technology, Daejeon and 3) Research and Development Center, Cell Biotech Co. Ltd., Gimpo, Republic of Korea

Abstract

We report the genome sequence of *Bifidobacterium animalis* subspecies *lactis* BL3, which has preventive properties on acute colitis and colon cancer. The genome of BL3, which was isolated from Korean faeces, consisted of a 1 944 323 bp size single chromosome, and its G+C content was 60.5%. Genome comparison against the closest *Bifidobacterium animalis* strain revealed that BL3 had particularly different regions of four areas encoding flavin-nucleotide-binding protein, transposase, multidrug ABC transporter and ATP binding protein.

Keywords: *Bifidobacterium*, genome sequence, IBD, PacBio, probiotics

Original Submission: 20 March 2017; Accepted: 11 May 2017

Article published online: 27 May 2017

Introduction

Inflammatory bowel disease (IBD) is a group of chronic inflammatory disorders occurring in the digestive tract [1]. Ulcerative colitis and Crohn disease, as principal types of IBD, can affect the entire gastrointestinal tract [2]. While ulcerative colitis affects the large intestine and rectum with continuous inflammation, Crohn disease causes inflammation of the lining of overall digestive tract and can even spread deep into tissue [3–5].

IBD symptoms include abdominal pain, vomiting, diarrhoea, rectal bleeding and weight loss [6,7]. Currently there are no drugs for the treatment of IBD and only few therapeutic options for modulating intestinal inflammation; sustained IBD can be an increased risk factor for colorectal cancer [2]. IBD a complex disease caused by various factors such as environment, genetics, immunologic responses and inflammation [8]. However, recent studies have paid attention to gut microbiota and have suggested that alterations of the intestinal microbiota may contribute to inflammation and the progression of IBD [9,10]. Therefore, modulation of intestinal flora could be a therapy for IBD treatment.

Previously we isolated *Bifidobacterium animalis* subspecies *lactis* BL3 strain from Korean faeces, which showed a preventive effect on acute colitis and colitis-associated colon cancer by inhibiting NF-κB activity [11]. In order to gain better insight into the preventive effects of probiotic *Bifidobacterium* on IBD, we analysed the genome sequence of *B. animalis* subspecies *lactis* BL3. Currently only seven genomes of *B. animalis* subspecies *lactis* strains are available, so the genetic information of this species is still insufficient. Therefore, in this study we analysed the whole genome sequence of *B. animalis* subspecies *lactis* BL3 to elucidate and understand preventive effect of probiotics on IBD and related disorders. Further characterization of genomic contents in probiotic *Bifidobacterium* such as *B. animalis* subspecies *lactis* BL3 will be needed to develop health-promoting probiotics.
Materials and methods

Bacteria strains and DNA preparation
Previously isolated *B. animalis* subspecies lactis BL3 from Korean faeces was cultivated on BL medium at 37°C for 18 hours in an anaerobic condition. Genomic DNA was extracted from the cultured bacterium with a QIAamp DNA Mini Kit (Qiagen, Germantown, MD, USA). The purity, quality and quantity of extracted DNA was examined by a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Fisher, Waltham, MA, USA) and Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA) respectively.

Genome sequencing, assembly and annotation
The whole genome of *B. animalis* subspecies lactis was sequenced by the PacBio RS II platform. A 20 kb DNA library, constructed according to the manufacturer’s instructions, was sequenced by single-molecule real-time sequencing technology with P6 DNA polymerase and C4 chemistry. A total of 1038 high-quality sequences (182 595 subreads) were obtained from the sequencing. The sequences were assembled using HGAP 3.0, and annotation was carried out with National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline [12] through the NCBI Genome submission portal (GenomeSubmit, http://ncbi.nlm.nih.gov). DNAPlotter [13] was used to draw the chromosome topology of this genome. Functional classification

---

FIG. 1. Circular map of genomic features of *Bifidobacterium animalis* subspecies lactis BL3 plotting seven tracks. Track 1 (light blue; outset): forward-stranded coding CDS. Track 2 (blue): reverse-strand coding CDS. Track 3 (light purple): rRNA including 5S, 16S and 23S. Track 4 (green): tRNA. Track 5 (red): CRISPR array. Track 6 (light green and purple): GC content. Track 7 (light green and purple): GC skew.
of the coding genes was performed using BLASTP search against the Clusters of Orthologous Groups (COGs) database [14,15]. The CRISPR region was detected using CRISPRFinder [16]. The genomic similarity between two genome sequences was estimated by the computation of orthologous average nucleotide identity (OrthoANI) using the Orthologous Average Nucleotide Genomic Similarity between two genome sequences was estimated as 99.98% via OrthoANI computation. Genomic differences between DSM 10140 and BL3 were found at four regions: 881 501~882 000, 1 305 501~1306 000, 1 309 501~1310 000 and 1 820 501~1821 000. The first region encodes two tRNA genes and a coding gene (locus_tag: BGL50_03705), which produces flavin–nucleotide binding protein. The second region has no annotation. The third one encodes a coding gene (locus_tag: BGL50_05610), which is annotated as ‘transposase.’ The last region encodes two coding genes (locus_tag: BGL50_07615 and BGL50_07620), which produce ‘multidrug ABC transporter’ and ‘ATP-binding protein.’

The genomic and comparative genomic analyses of probiotic microorganisms will provide valuable information on the detailed functional properties and genotype-level safety of probiotics. The genetic study on the current probiotic microorganism will increase our knowledge of their biologic mechanisms against preventing human diseases and may lead to genome-based biotechnologic applications in the human healthcare and food industries utilizing Bifidobacterium strains.

**Nucleotide sequence accession number**

The assembled and annotated genome of *B. animalis* subspecies *lactis* BL3 has been deposited at DNA Data Bank of Japan, European Molecular Biology Laboratory, and GenBank under accession number CP017098. The version described here is the first version, CP017098. The genome sequence data are available in FASTA, annotated GenBank flat file and ASN.1 formats. This strain has also been deposited in the Korea Collection for Type Culture (KCTC 11904BP).

**Acknowledgements**

This research was supported by Main Research Program of the Korea Research Food Institute funded by the Ministry of Science, ICT and Future Planning (E0170602-01).

**Conflict of Interest**

None declared.

**References**

[1] Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet 2007;369:1641–57.

[2] Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol 2010;28:573–621.
[3] Lockhart-Mummery HE, Morson BC. Crohn’s disease (regional enteritis) of the large intestine and its distinction from ulcerative colitis. Gut 1960;1:87–105.

[4] Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology 2011;140:1704–12. e1702.

[5] Ford AC, Moayyedi P, Hanauer SB. Ulcerative colitis. BMJ 2013;346:f332.

[6] Hanauer SB, Sandborn W. Management of Crohn’s disease in adults. Am J Gastroenterol 2001;96:635–43.

[7] Liu S, Ren J, Zhao Y, Han G, Hong Z, Yan D, et al. Nonthyroidal illness syndrome: is it far away from Crohn’s disease? J Clin Gastroenterol 2013;47:153–9.

[8] Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. Lancet 2007;369:1627–40.

[9] Mukhopadhya I, Hansen R, El-Omar EM, Hold GL. IBD—what role do Proteobacteria play? Nat Rev Gastroenterol Hepatol 2012;9:219–30.

[10] Aroniadis OC, Brandt LJ. Fecal microbiota transplantation: past, present and future. Curr Opin Gastroenterol 2013;29:79–84.

[11] Kim SW, Kim HM, Yang KM, Kim SA, Kim SK, An MJ, et al. Bifidobacterium lactis inhibits NF-κB in intestinal epithelial cells and prevents acute colitis and colitis-associated colon cancer in mice. Inflamm Bowel Dis 2010;16:1514–25.

[12] Tatusova T, Dicuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, et al. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 2016;44:6614–24.

[13] Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J. DNAPlotter: circular and linear interactive genome visualization. Bioinformatics 2009;25:119–20.

[14] Tatusov RL, Galperin MY, Natale DA, Koonin EV. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res 2000;28:33–6.

[15] Clarke GP, Beiko RG, Ragan MA, Charlebois RL. Inferring genome trees by using a filter to eliminate phylogenetically discordant sequences and a distance matrix based on mean normalized BLASTP scores. J Bacteriol 2002;184:2072–80.

[16] Grissa I, Vergnaud G, Pourcel C. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 2007;35:W52–7.

[17] Lee I, Kim YO, Park SC, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.

[18] Laing C, Buchanan C, Taboada EN, Zhang Y, Kropinski A, Villegas A, et al. Pan-genome sequence analysis using Panseq: an online tool for the rapid analysis of core and accessory genomic regions. BMC Bioinformatics 2010;11:1.