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GENOME ANNOUNCEMENT

Complete Genome Sequence of the Probiotic Bacterium *Bifidobacterium bifidum* Strain BGN4

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***Bifidobacterium bifidum*, a common endosymbiotic inhabitant of the human gut, is considered a prominent probiotic microorganism that may promote health. We completely decrypted the 2.2-Mb genome sequence of *B. bifidum* BGN4, a strain that had been isolated from the fecal sample of a healthy breast-fed infant, and annotated 1,835 coding sequences.**

Bifidobacterium bifidum BGN4, a β-glucosidase-negative strain that was isolated from the fecal sample of a healthy, breast-fed infant, first drew our special attention because the β-glucosidase activity in the intestine can produce carcinogenic or mutagenic aglycones from various glycosides such as rutin, guercitrin, robinin, and cycasine (3). As probiotics responsible for intestinal healthiness, there are several lines of evidence from *in vitro* and *in vivo* experiments supporting the notion that bifidobacteria can modulate the host immune system and inhibit pathogen infection (11). In particular, the anticarcinogenic polysaccharide isolated from the cytosolic fraction of BGN4 inhibits the growth of some cancer cell lines (19) and it was also reported to have a potent adhering activity with respect to Caco-2 cells and to be able to alleviate allergic reactions elicited by ovalbumin in a mouse model (9, 10).

The genome sequence was determined by the use of a Roche GS FLX system (NICEM, Republic of Korea). A total of 209,020 reads totaling up to 23.18× coverage were assembled into 27 contigs using GS Assembler. Gap closing was performed by multiplex PCR and primer walking on the amplified products by the standard Sanger sequencing. Sequence manipulation, primer design, and manual validation were performed using Phred/Phrap/CONSED (6). Protein-coding genes were predicted by the combination of CRITICA (2) and GLIMMER (4). tRNAs and rRNAs were identified by tRNAScan-SE (15) and BLAST (1), respectively. All predicted genes were annotated by AutoFACT (12), with additional searches performed using the TIGRFAMs database (18) and protein sequences from the genomes of *B. longum* species (13, 17) and *B. adolescentis* ATCC 15703.

The complete sequence consists of a 2,223,664-bp circular chromosome (62.65% G+C) with no plasmid. We compiled 1,835 coding sequences (CDSs), 7 pseudogenes, 3 rRNA operons, and 52 tRNAs from the nucleotide sequence. A total of 1,373 CDSs were assigned predicted functions, while the rest was designated conserved hypothetical proteins or hypothetical proteins. The genome contains 27 insertion sequence elements or transposons and 20 kinds of aminoacyl-tRNA synthetase genes. In particular, a BGN4-specific 52-kb segment (bp 1392576 to 1445526) encoding two mobilization proteins (MobC [BBB_1196] and MobA [BBB_1198]), 16 functional proteins, and 28 hypothetical proteins was identified by genomewise comparison with *B. bifidum* PRL2010, which might have been acquired by horizontal gene transfer.

The genome sequence analysis helps elucidate the phenotypic features of BGN4, including its probiotic effects. For example, the gene encoding glutamine fructose-6-phosphate amidotransferase (GlmS [BBB_0791]) that is involved in *N*-acetylglucosamine biosynthesis is interrupted by a stop codon to make it a pseudogene, which might be responsible for the *N*-acetylglucosamine auxotrophy of *B. bifidum* (5). Moreover, the presence of a homolog (BBB_0596) of the bifidobacterial outer protein (BopA) (7) suggests its high capacity for adhesion to the Caco-2 cell line. Deconjugation of bile salts and the reduction of serum cholesterol levels are closely related (14, 16), and BBB_0854, homologous to bile salt hydrolase (EC 3.5.1.24), may contribute to bile salt tolerance.

Nucleotide sequence accession number. Genome sequence information was registered in GenBank under accession number CP001361. The sequence and annotation are also available from the Genome Encyclopedia of Microbes (GEM; https://www.gem.re.kr) (8).

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REFERENCES

1. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.
2. **Badger JH, Olsen GJ.** 1999. CRITICA: coding region identification tool invoking comparative analysis. Mol. Biol. Evol. **16**:512–524.
3. **Choi YJ, et al.** 1996. Growth and β-glucosidase activity of *Bifidobacterium*. J. Microbiol. Biotechnol. **6**:255–259.
4. **Delcher AL, Harmon D, Kasif S, White O, Salzberg SL.** 1999. Improved

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microbial gene identification with GLIMMER. Nucleic Acids Res. **27**: 4636–4641.
5. **Foley S, et al.** 2008. Characterisation of glutamine fructose-6-phosphate amidotransferase (EC 2.6.1.16) and *N*-acetylglucosamine metabolism in *Bifidobacterium.* Arch. Microbiol. **189**:157–167.
6. **Gordon D, Abajian C, Green P.** 1998. Consed: a graphical tool for sequence finishing. Genome Res. **8**:195–202.
7. **Guglielmetti S, et al.** 2008. Implication of an outer surface lipoprotein in adhesion of *Bifidobacterium bifidum* to Caco-2 cells. Appl. Environ. Microbiol. **74**:4695–4702.
8. **Jeong H, Yoon SH, Yu DS, Oh TK, Kim JF.** 2008. Recent progress of microbial genome projects in Korea. Biotechnol. J. **3**:601–611.
9. **Kim H, Kwack K, Kim DY, Ji GE.** 2005. Oral probiotic bacterial administration suppressed allergic responses in an ovalbumin-induced allergy mouse model. FEMS Immunol. Med. Microbiol. **45**:259–267.
10. **Kim IH, Park MS, Ji GE.** 2003. Characterization of adhesion of *bifidobacterium* sp. BGN4 to human enterocyte-like caco-2 cells. J. Microbiol. Biotechnol. **13**:276–281.
11. **Kim JF, et al.** 2009. Genome sequence of the probiotic bacterium *Bifidobacterium animalis* subsp. *lactis* AD011. J. Bacteriol. **191**:678–679.
12. **Koski LB, Gray MW, Lang BF, Burger G.** 2005. AutoFACT: an automatic functional annotation and classification tool. BMC Bioinformatics **6**:151.
13. **Lee JH, et al.** 2008. Comparative genomic analysis of the gut bacterium *Bifidobacterium longum* reveals loci susceptible to deletion during pure culture growth. BMC Genomics **9**:247.
14. **Liong MT, Shah NP.** 2005. Bile salt deconjugation ability, bile salt hydrolase activity and cholesterol co-precipitation ability of lactobacilli strains. Int. Dairy J. **15**:391–398.
15. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**: 955–964.
16. **Noriega L, Cuevas I, Margolles A, de los Reyes-Gavilán CG.** 2006. Deconjugation and bile salts hydrolase activity by *Bifidobacterium* strains with acquired resistance to bile. Int. Dairy J. **16**:850–855.
17. **Schell MA, et al.** 2002. The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. Proc. Natl. Acad. Sci. U. S. A. **99**:14422–14427.
18. **Selengut JD, et al.** 2007. TIGRFAMs and Genome Properties: tools for the assignment of molecular function and biological process in prokaryotic genomes. Nucleic Acids Res. **35**:D260–D264.
19. **You HJ, Oh DK, Ji GE.** 2004. Anticancerogenic effect of a novel chiroinositol-containing polysaccharide from *Bifidobacterium bifidum* BGN4. FEMS Microbiol. Lett. **240**:131–136.