

# 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  $\beta$ -glucosidase-negative strain that was isolated from the fecal sample of a healthy, breast-fed infant, first drew our special attention because the  $\beta$ -glucosidase activity in the intestine can produce carcinogenic or mutagenic aglycones from various glycosides such as rutin, guercitrin, robinin, and cysanine (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 $\times$  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 genomewide 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 homology of 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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