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Development of *Bifidobacterium* sp. BGN4 and BORI with Novel Probiotic Activity

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Bifidobacterium spp. is nonpathogenic, gram-positive and anaerobic bacteria which inhabit the intestinal tracts of humans and animals. In breast-fed infants, bifidobacteria comprise more than 90% of the gut bacterial population. *Bifidobacterium* strains are used in commercial fermented dairy products and various probiotic products and have been suggested to exert health promoting effects on the host by maintaining intestinal microflora balances, improving lactose tolerance, increasing synthesis of vitamins, and aiding the immune enhancement and anticarcinogenic activity for the host. These beneficial effects of *Bifidobacterium* are known to be strain-specific. We report the development of *Bifidobacterium* sp. BGN4 and BORI with novel probiotic features.

Selection and characterization of BGN4 with high adhesive ability to human enterocyte-like cells

The adhesion of probiotic bacteria to the intestinal mucosa is one of the desirable properties for their colonization in the intestinal tract where these bacteria constantly compete with other bacteria. The adhesion of different strains of bifidobacteria to Caco-2 cells was compared. Among them, strain BGN-4 showed the highest adhesion. Upon protease and heat treatment the adhesion of BGN-4 to the Caco-2 cells decreased significantly. The cells grown at 42℃ showed less self-aggregation than cells grown at 37℃. The treatment with EGTA did not have any effect on the adhesion. The degree of adhesion did not differ between the experimental groups when galactose, mannose and fucose are added in the adhesion assay. The results suggest that the adhesion of the *Bifidobacterium* to the epithelial cells may be affected by the composition and structure of the cell membrane and interacting surfaces.

Chiro-inositol based polysaccharide with anti-tumor activity from *Bifidobacterium bifidum* BGN4

In our laboratory a selected strain *B. bifidum* BGN4 showed a strong adhesion to a human enterocyte cell line, Caco-2, and anti-tumor effect in the in-vitro and in-vivo animal models. A novel anti-tumor bioactive compound was purified and identified as a polysaccharide (BB-pol) containing chiro-inositol as a major component of the BB-pol. BB-pol showed a considerable anti-tumor effect on the HT-29 and HCT-116 cell lines in a dose-dependent manner. The concentration which inhibited cell growth was about 20μg/ml measured by trypan blue exclusion assay and BrdU incorporation assay. DNA microarray was performed using a human 10K oligonucleotide chip containing 10,108 human genes to analyze the transcriptional responses after BB-pol treatment. BB-pol treatment changed the expression of 154 and 254 genes in HT-29 and HCT-116 cells, respectively. Among these, 62 genes were down-regulated and 13 genes were up-regulated in both cell lines. Twenty four genes among these 75 genes belonged to protein-tyrosine kinases (PTK), protein-tyrosine phosphatases (PTPase), signal transduction-related genes, transcriptional regulators, and transporters, etc. Tumor suppressor genes such as TGFBR2 and BIN1 were upregulated after BB-pol treatment. Further illustration of the presently identified genes may give more detailed picture how BB-pol from a probiotic *B. bifidum* BGN4 inhibit the growth of colon cancer cell lines.

Viability and intactness of *Bifidobacterium* sp. BGN4 in the effects of allergy suppression

The effects of cell viability and intactness of *Bifidobacterium* on the suppression of allergy was investigated. C3H/HeJ mice were sensitized at weeks 3, 4, 6 and 8 with ovalbumin and choleratoxin to induce allergic reaction. Mice fed with 0.2% of live, disrupted or heat-killed *Bifidobacterium bifidum* BGN4 in the pellet of diet for 8 weeks starting 2 weeks before initial sensitization differentially suppressed the allergy response in terms of levels of IgE and IgG1 in sera, and symptoms on tail. Viable *Bifidobacterium* was more effective than disrupted or heat-killed cells in the suppression of allergy. Growth inhibition, which occurred in the sham group at week 4, was not shown in *Bifidobacterium* treated groups. These results show that *Bifidobacterium* exhibits suppressive effect on allergy response of mice, and viability and intactness of *Bifidobacterium* is required for the effective suppression of allergy.

Effective timing of *Bifidobacterium* sp. BGN4 administration for the suppression of ovalbumin allergy in mice

We evaluated the effect of timing of probiotic administration on food allergy in an *in vivo* ovalbumin-induced food allergy mouse model. Being sensitized with ovalbumin (OVA) and choleratoxin (CT) for 5 weeks, C3H/HeJ mice were administered *Bifidobacterium bifidum* BGN4 continuously for 2 weeks before (pre-treatment group) and after (post-treatment group) initial sensitization. After sensitization, the OVA-induced (sham group) mice had high levels of OVA specific IgE and IgG1 in sera, as well as scab-covered tails. The pre-treatment group had lower levels

of OVA specific IgE and IgG1 in sera, as well as less severe tail symptoms. The post-treatment group had lower levels of OVA specific IgE but unchanged levels of IgG1 and IgG2a. In spleen cultures, levels of IFN-γ were higher in the pre-treatment and post-treatment groups than in the sham group, while levels of IL-6 and IL-18 were lower in the pre-treatment and post-treatment groups than in the sham group. Growth inhibition occurred in the sham group but not in the pre-treatment and post-treatment groups. Thus, treatment with *Bifidobacterium* before OVA sensitization appears to suppress or modulate food allergy more effectively than treatment with *Bifidobacterium* following OVA sensitization.

Purification of anti-rotavirus protein from *Bifidobacterium longum* BORI

Rotavirus is the major causative infectious agent of the diarrhea in children during winter and is estimated to cause more than 800,000 annual deaths of young children in developing countries. Several lactic bacteria strains are reported to reduce the duration and severity of symptoms from rotavirus-associated diarrhea. However, the mechanism of action is still not elucidated. In our experiment we isolated a strain which showed a superior anti-rotavirus effect to other various *Bifidobacterium* strains and named the strain as *B. longum* BORI. Administration of *B. longum* BORI to the rotavirus antibody bearing children with acute colitis significantly lowered the incidence of diarrhea compared to the non-treated control group. A protein with anti-rotavirus activity was purified to homogeneity from *B. longum* BORI and showed anti-rotavirus activity with IC at 2 ng/ml in *in-vitro* cell line model. Its corresponding gene was cloned and expressed both in *E. coli* and *Bifidobacterium* genetic vector system.

Effect of *Bifidobacterium* sp. BGN4 intake on the composition of human large intestinal bacteria in the elderly

Effect of dietary supplementation of *Bifidobacterium* sp. BGN-4 on the composition of large intestinal bacteria of the elderly people was investigated. Four grams of supplements containing 10^9 cfu/g *Bifidobacterium* sp. BGN4 were administered daily to 14 elderly volunteers every day for 10 days, followed by 10 days of non-intake period, and the cycle was repeated for 50 days. Composition of the intestinal bacteria (*Bacteroides, Bifidobacterium, Lactobacillus, E. coli, Clostridium perfringens*) examined revealed that administration of *Bifidobacterium* sp. BGN4 resulted in a marked increase in *Bifidobacterium* and a decrease in *Bacteroides.* Stool evacuation frequencies, pH, and water contents of the fecal samples did not change significantly.

Effect of *Bifdobacterium* sp. BORI on the severity in rotavirus-infected children

Anti-rotavirus positive children patients who were admitted to the Yonsei University Hospital were enrolled in the study. The patients were randomly assigned into two groups: the probiotics group

receiving *Bifidobacterium longum* BORI and *Lactobacillus acidophilus* twice a day and the placebo group. The average age of the participants were 17.8 month in both groups. Sex and serological measurements did not differ between the two groups. The stool frequencies were significantly decreased by 3-day administration of probiotics. The period of fever, diarrhea and vomiting were also reduced even though not at a significant level. The results suggest that probiotic treatment reduced the severity of the clinical symptoms in the rotavirus-infected children.