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Development of *Bifidobacterium* with Improved Probiotic Activity

Myeong-Soo Park[1], Hyun-Ju You[2], Hye-Young Kim[2] and Geun-Eog Ji[1,2]*

[1]Dept. of Food and Nutrition, Seoul National University
[2]Bifido Research Institute; E-mail: geji1@naver.com

Bifidobacterium spp. are 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 have been suggested to exert health promoting effects on the host by maintaining intestinal microflora balances, improving lactose tolerance, reducing serum cholesterol levels, increasing synthesis of vitamins, and aiding the immune enhancement and anticarcinogenic activity for the host (1-4). These beneficial effects of *Bifidobacterium* are known to be strain-specific. Therefore continued efforts to improve strain characteristics are desired.

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 (5), Caco-2, and anti-tumor effect in in-vitro and in-vivo animal models (6). 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 (7). BB-pol showed a considerable anti-tumor effects on the HT-29 and HCT-116 cell lines in a dose-dependent manner. The polysaccharide fraction which contained BB-pol was extracted from *B. bifidum* BGN4, and treated to the human colon cancer cell lines (7). BB-pol's growth inhibitory effects were cell line specific (especially HT-29 and HCT-116 cells), and the minimum concentration which inhibited cell growth was about 20 μg/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.

The Effect of Oral Feeding of *B. bifidum* BGN-4 in a Murine Model of Food Allergy

Food allergy has been increasing in prevalence especially in well-developed countries (8,9). The so-called hygiene hypothesis attributes the increasing prevalence of atopic diseases in industrialized areas to disease reduction resulting from vaccinations and improved hygiene in western countries (10). Despite of the potential for a fatal outcome, no definitive therapies are available for food allergy and allergen avoidance is the only therapeutic option. Recently, some strains of probiotic bacteria are reported to ameliorate the occurrence of food

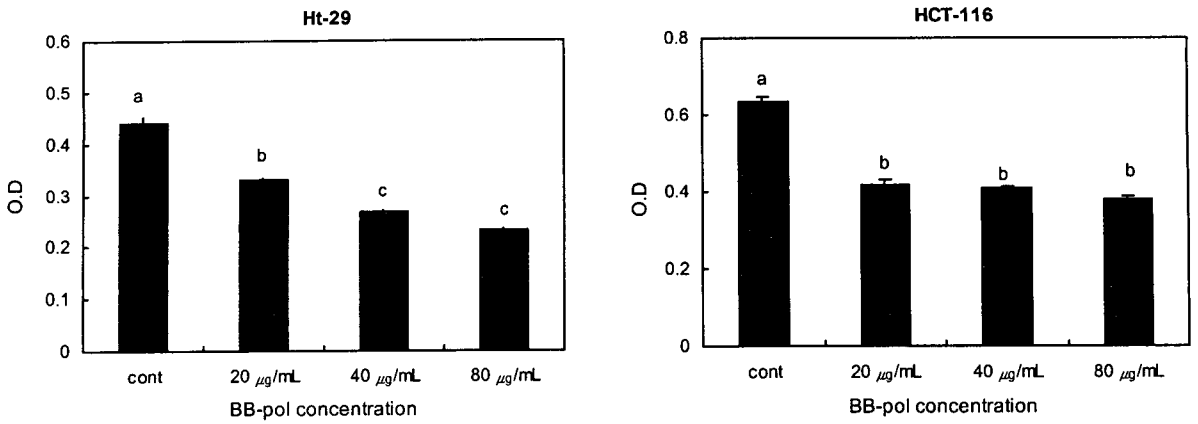

Fig. 1. Effect of BB-pol on the growth inhibition of HT-29 and HCT-116 cell lines measured by BrdU incorporation assay. The cells were cultured for 48 h in the presence of BB-pol at various concentrations in 96-well plates: 0 (control), 20, 40, and 80 μg mL^{-1}. The DNA synthesis rates were determined by BrdU incorporation assay. Data values are mean± SE of triplicates in a representative assay ($p<0.0001$).

Table 1. The effect of *Bifidobacterium* sp. BGN4 on number and size of abberant cript foci (Shim, 2002)

Group	ACF	Size
Control	105	+++++
BGN4	35	++

allergies in human clinical studies and animal models. In our study (11), we examined the inhibitory effect of *B. bifidum* BGN4 on IgE production in ovalbumin (OVA)- and peanut antigen-sensitized C3H/HeJ mice and in vitro cell culture experiment. Sera from sensitized C3H/HeJ mice were collected weekly. We also examined the pattern of cytokine production by spleen cells from mice fed BGN-4 followed by re-stimulation with sensitizing antigen in vitro in the presence of heat-killed BGN-4. In the mice fed BGN-4, OVA- and peanut antigen-specific Immunoglobulin E was increased at a significantly lower level than in the control allergy group. Also we noticed that mice of allergy group frequently scratched their tails whereas mice fed BGN-4 did not. In the spleen cells, the production of Th1 cell-associated cytokines such as interferon-gamma, immunoglobulin G2a and interlukin-2, were higher in the mice BGN-4 than in the allergy group. In contrast, the production of interlukin-12 and TH2 cell-associated cytokines, such as interlukin-4, interlukin-6 and interlukin-10 was lower in the mice fed BGN-4 than that in the allergy group. In conclusion, oral feeding of *B. bifidum* BGN4 showed a preventive effect on the occurrence of IgE mediated allergy. The increased Th1 cell-associated cytokines by BGN-4 may play an important role on the prevention of allergy

Purification of anti-rotavirus protein from *B. 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 (12). A rotavirus vaccine developed by Wyeth Company was approved by FDA in 1999 but its approval was cancelled thereafter since it showed considerable side effects. Currently no effective drug or vaccine against rotavirus is available in the market. 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 (13). In our experiment we isolated a strain, which showed a superior anti-rotavirus effect to other various *Bifidobacterium* strains and named the

Table 2. Clinical treatment of children with acute diarrhea using *Bifidobacterium longum* BORI

	Treated[1)]	Untreated
Age (Month)	16.4±8.5	19.1±11.1
Diarrhea period (day)	4.38±1.29	5.61±1.23
Pyrexia period (day)	3.66±1.14	4.32±1.94
Diarrhea number (time/day)	2.38±0.49	2.64±0.73
Emesis period (day)	1.55±1.12	1.82±0.94

[1)]Treatment dosage was 4.0×10^9 CFU/day.

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 0.045 μg/mL in a in vitro cell line model. Its corresponding gene was cloned and expressed in *E. coli* genetic vector system.

Development of *Bifidobacterium* Genetic Vector System

Genetically engineered useful traits using *Bifidobacterium* vectors would be extremely useful in improving the health state of the large-intestinal tract if the ethical and public view of the use of genetically engineered organism is accepted. *Bifidobacterium* is the most promising in serving as a delivery system for the useful gene products into human intestinal tract (14). The application of genetically engineered *Bifidobacterium* would also allow the manufacture of superior quality probiotic food products. Our lab has isolated and sequenced several *Bifidobacterium* plasmids including pKJ 50, pKJ 36 and pMG1 (15-17). Using the plasmid (pMG1) a novel *E. coli-Bifidobacterium* shuttle vector pBES2 was constructed. Also, we have cloned and sequenced several metabolic genes and genetic promoters from *Bifidobacterium* which could be good candidates for gene expression controller and food grade selection marker. For the expression and secretion of foreign genes, a construct pYBamy59 which contained an amylase gene inserted into pBES2 was made. Both *Bifidobacterium* as well as *E. coli* transformants harboring pYBamy59 expressed and secreted amylase into the medium. Using our *Bifidobacterium* vectors several genes coding for such as cholesterol oxidase, phytase, endostatin and glutamate decarboxylase were expressed. Now we are in a very exciting stage for the expression of pharmacologically and functionally important gene products using genetic vector system in probiotic *Bifidobacterium* strains.

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