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RESEARCH NOTE

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Effect of *Bifidobacterium*-fermented Soy Hypocotyls Intake on the Composition of Human Large Intestinal Bacteria in the Elderly

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Abstract Effect of dietary supplementation of *Bifidobacterium*-fermented soy hypocotyls (BFSH) on the composition of large intestinal bacteria of the elderly people was investigated. Four grams of BFSH containing 10^9 cfu/g *Bifidobacterium* 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 BFSH 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.

Keywords: *Bifidobacterium*, soy, intestinal bacteria, elderly

Introduction

The human intestinal tract contains a complex and dynamic bacterial population, whose cell numbers amount to 10^{14} cfu (colony forming units) per gram (1). The metabolic activities of the bacteria can exert both harmful or beneficial effects on the human host depending on the species, diet, and other various environmental factors (1). The number of putrefactive bacteria such as *Clostridium perfringens* are known to increase with the aging of the human host, leading to increased production of ammonia, H_2S, and amines, which can aggravate the intestinal environment of the elderly people (2). On the other hand, the number of beneficial bacteria such as *Bifidobacterium* decreases at a significant level during aging. Upon examining the composition of the intestinal bacteria of the Koreans, Ji reported that number of *Bifidobacterium* was lower and that of *Cl. perfringens* higher in the elderly people (above 65 years group) than the young people (3). In spite of the recent marked increase in elderly people in Korea, little studies have been conducted to improve the health state in terms of the intestinal bacterial balance for the elderly people. Probiotics are well known to affect the composition of indigenous microflora and may have several beneficial effects on the human health such as the maintenance of a balanced flora, alleviation of lactose intolerance symptom, and resistance to enteric pathogens (4). Modes of action of probiotics are suggested to be the colonization of the gastro-intestinal tract, prevention of pathogen overgrowth, neutralization of enterotoxins, modulation of the activity of bacterial enzymes in the large intestine, improvement of the digestive capacity of the small intestine, and adjuvant effect on the immune system (5). In most cases, clinical studies were preceded by laboratory-based research, utilizing animal models or *in vitro* culture systems, to provide preliminary evidence on the intestinal condition. In this study, soybean hypocotyls, part of the axis of soybean embryo below the cotyledons, were fermented with *Bifidobacterium*. The effect of dietary supplementation of *Bifidobacterium*-fermented soy hypocotyls (BFSH) on the intestinal bacteria of the elderly people was investigated.

Materials and Methods

Soybean hypocotyls were ground and suspended in 10 volumes of water. The soy hypocotyls medium was flushed with gas mixture (95% N_2 and 5% CO_2) to remove dissolved oxygen. For fermentation, *Bifidobacterium* sp. BGN4 previously characterized (6) was used. After autoclaving, fresh *Bifidobacterium* sp. BGN4 cultures were inoculated at a 1:50 ratio. The suspension fermented for 24 hr was lyophilized until dryness. It contained *Bifidobacterium* counts of 10^9cfu/g dry weight. Four grams (two grams after breakfast and two grams after dinner) of the powder were administered to 14 elderly volunteers (7 each males and females) every day for 10 days, followed by 10 days of non-intake period. The nonintake-intake cycle was repeated for 50 days. During the study period, all subjects were asked to avoid consumption of fermented food and other probiotic products. The composition of intestinal bacteria was examined at the last day of each period using fresh fecal samples. The serially diluted samples were plated on various selective and non-selective media as described by Ji (3). For counting anaerobic organism, Anoxomat (MART, Netherland) was used to equilibrate

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Table 1. Effect of BFSH intake on the composition of human feces

	Non-intake / Intake cycle				
	Before intake	First intake	First non-intake	Second intake	Second non-intake
Microorganism	(Log of CFU per gram wet feces)				
Bifidobacterium	9.0±0.5	9.5±0.5	9.0±0.8	10.0±0.4	9.1±0.8
Bacteroides	9.6±0.4	8.7±1.5	10.1±0.4	9.7±0.3	9.9±0.6
Lactobacillus	ND	7.2±1.4	7.5±1.3	8.0±1.4	7.5±1.2
Clostridium	4.5±0.7	4.8±1.3	5.1±0.6	4.7±0.9	5.3±1.1
E. coli	8.6±0.5	7.6±1.0	7.8±1.0	7.9±2.3	7.6±0.8
Total aerobic bacteria	8.8±0.4	7.5±1.0	8.1±0.8	8.4±0.6	8.4±0.5
Water content (%)	73.6±8.6	70.2±9.1	70.2±8.5	71.7±9.2	73.2±8.9
pH	6.9±0.6	6.8±0.8	6.8±0.9	6.9±1.0	6.5±0.6

All numbers in Mean±S.D. ND means not determined.

the anaerobic chamber and the plate was incubated 2-3 days at 37°C. The pH was measured by a pH meter (Fisher Model 10, USA) after the suspension of fecal samples with 4 volumes of water. The moisture contents were analyzed after drying at 105°C in a drying oven. Group means comparisons were tested for significance by Student's *t*-test. Statistical significance was defined as $P<0.05$. Fecal composition results of the subjects are expressed as Means±S.D..

Results and Discussion

Bioconversion of soy oligosaccharides and isoflavones, and their corresponding enzymes, alpha-galactosidase and beta-glucosidase, has been previously characterized in our laboratory during soy fermentation using *Bifidobacterium* (7, 8). For the present study, we used a hypocotyl portion of the soy for fermentation. The effect of dietary supplementation with BFSH on intestinal bacteria of the elderly people is shown in Table1.

Among the examined bacteria, viable *Bifidobacterium* cell number showed the most pronounced increase during intake, whereas that of *Bacteroides* decreased following the consumption of BFSH. No significant changes were obtained for *E, coli, Lactobacillus,* and *Cl. perfringens*. *Bifidobacterium* comprising more than 90% of the intestinal bacteria in breast-fed infants decreases down to about 10% after weaning and during the following life time period (1). *Bifidobacterium* has been considered to confer various beneficial effects on the human hosts, whereas *Bacteroides* are known to produce various putrefactive compounds and are the most frequently found anaerobic infectious agents in various tissues aside from the intestine (1, 2). In this context, our result may be interpreted as an improvement in the balance of the intestinal bacteria by the supplementation of BFSH. Further analysis needs to be performed on whether the observed effects were solely due to administered *Bifidobacterium* or whether some other components of BFSH are responsible. The effect of BFSH intake on pH, moisture contents, and stool evacuation frequencies were not eminent. Throughout the experiment, the participants showed a high interest in the experiments and willingness to donate their fecal samples. More research on the development of the probiotic foods for the elderly is warranted considering that the population of the elderly will soon double by year 2020. Further studies should delineate the mechanism of the probiotic action, the evaluation of the physiological effect, and the efficient delivery method of probiotic products for special targets (e.g., age group, intestinal organ, etc.) in more detail.

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