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Protective Effects of *Bifidobacterium* spp. on Experimental Colon Carcinogenesis with 1,2-Dimethylhydrazine

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Abstract The protective role of *Bifidobacterium* spp. (*B. breve* K-110, *B. breve* K-111, and *B. infantis* K-525) isolated from the fecal samples of healthy Koreans was investigated on 1,2-dimethylhydrazine (DMH)-induced aberrant crypt foci (ACF) formation in mouse colon. In mice fed normal diet with DMH treatment, an average of 68.5 ACF/colon was formed, whereas in mice administered with *B. breve* K-110, *B. breve* K-111, and *B. infantis* K-525, the numbers of DMH-induced ACF decreased to 7.2, 10.9, and 6.6 ACF/colon, respectively. The mean number of crypts/focus was not significantly altered. Fecal harmful enzymes, such as β-glucuronidase, tryptophanase, and urease, were effectively inhibited during the administration of these bifidobacteria to mice. These results suggest that bifidobacteria could prevent colon cancer.

Key words: β-glucuronidase, *Bifidobacterium* spp., colorectal cancer, aberrant crypts

Epidemiological studies suggest that dietary factors, such as high animal fat and protein, are the prime factors in the etiology of colon cancer [2, 12]. It has been suggested that the high intake of these dietary factors may cause a high excretion of bile acids and cholesterol, which are converted by intestinal bacteria to secondary bile acids and fecal sterols. These bacteria may be further metabolized to steroid carcinogens [3, 9]. Dietary factors may also alter the bacterial flora of the intestine and their enzymatic activities (β-glucuronidase and nitroreductase), and thereby influence the metabolism of fecal sterols or exogenous carcinogens [13]. The carcinogen 1,2-dimethylhydrazine (DMH) can induce colorectal adenocarcinoma as well as β-glucuronidase of intestinal bacteria. Intestinal bacteria may play an important role in liberating active chemical carcinogens, inducing colon tumors [8]. When DMH is subcutaneously injected into the rat, it is conjugated with glucuronic acid immediately in the liver and excreted to the intestine via the bile duct. This excreted glucuronic acid conjugate would be hydrolyzed by bacterial β-glucuronidase to free compound. A relatively high concentration of this compound in the colon may cause the colonic adenocarcinoma [1]. Therefore, DMH-induced colorectal adenocarcinoma may be prevented if fecal β-glucuronidase could be inhibited.

Lactic acid bacteria have been considered as the most beneficial probiotic organism contributing to the inhibition of harmful and putrefactive intestinal bacteria, and improve lactose malabsorption in humans, as well as leading to the enforcement of immune functions and prevention of cancer [11]. Among them, *Bifidobacterium* spp. has been considered as one of the most beneficial probiotic organisms improving human health, since it is one of the major bacterial flora in the human intestine and has various kinds of biological activities. We screened *Bifidobacterium* spp. inhibiting harmful enzymes of human intestinal microflora and isolated three bifidobacteria, *B. breve* K-110, *B. breve* K-111, and *B. infantis* K-525, from healthy Koreans. These strains showed the most inhibitory activity *in vitro* on harmful enzymes in human intestinal microflora [6, 10].

Here, we investigated the *in vivo* inhibitory effects of *Bifidobacterium* spp. on harmful enzymes of intestinal microflora and the protective effects of these species on experimental colon carcinogenesis with DMH.

Culture of Lactic Acid Bacteria

B. breve K-110, *B. breve* K-111, and *B. infantis* K-525, which were isolated from fecal samples of the healthy Korean subjects as lactic acid bacteria with potent biological activities, were inoculated into general anaerobic medium (Nissui Pharm. Co. Ltd., Japan), respectively. Then, each cultured bacteria was inoculated into 500 l of tryptic soy broth containing 0.05% sodium thioglycolate and 0.5% ascorbic acid. The cultured bacteria were centrifuged at 4500×g for 20 min. The precipitate was washed with saline and then used as a sample.

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Animal Study

In all studies, the animals were housed in plastic cages with wire tops. Unless otherwise stated, the animals were fed mouse diet (Samayang Co., Korea) *ad libitum* and had free access to water. For the *in vivo* inhibitory activity assay of the *Bifidobacterium* spp. on some of the harmful enzymes in mice intestinal bacteria, the mice (ICR males, 15 g) were divided into four groups [control group (I), *B. breve* K-110-treated group (II), *B. breve* K-111-treated group (III), and *B. infantis* K-525-treated group (IV)]. Ten animals in each group were used. Group I was fed the usual powdered laboratory diet. Groups II, III, and IV were fed the usual powdered laboratory diet containing 0.5% *B. breve* K-110, *B. breve* K-111, and *B. infantis* K-525, respectively. The activities of fecal enzymes (β-glucosidase, β-glucuronidase, tryptophanase, and urease) and the concentration of ammonia were assayed every week according to the method of Kim *et al.* [6, 4].

To study the protective activity of DMH-induced colon carcinogenesis, the mice (ICR males, 15 g) were divided into 5 groups of ten animals each. Four groups [DMH-treated group (II), DMH and *B. breve* K-110-treated group (III), DMH and *B. breve* K-111-treated group (IV), DMH and *B. infantis* K-525-treated group (V)] were given weekly s.c. injection of DMH (20 mg/kg of body weight per week) for 10 weeks. The other group [control group (I)] was given weekly s.c. injections of saline not containing DMH. Group I and Group II were fed the usual powdered laboratory diet (10 g/mouse/day). Groups III and VI were fed the powdered diet containing 0.5% *B. breve* K-110. Groups IV and VII were fed the powdered diet containing 0.5% *B. breve* K-111. Groups IV and VIII were fed the powdered diet containing 0.5% *B. infantis* K-525. After the last injection, all animals were fed for 5 weeks and then sacrificed.

Visualization and Quantification of Aberrant Crypts (AC)

The animals were sacrificed randomly with diethylether 16 weeks after the initiation of the experiment. The colon was removed immediately, flushed with Kreb's Ringer solution, slit open from caecum to anus, and fixed in 10% buffered formalin. Following the published protocol [5, 7], the colons were fixed, stained with methylene blue, and assessed for AC by using the light microscope. Parameters used to assess AC were their occurrence and distribution. The occurrence was measured by quantitating the mean number of AC foci (ACF) per colon. The number of AC per focus was also recorded. To determine the distribution of AC, the colon was divided into two sections. The rectum represented the first 2 cm from the rectal end. The sigmoidal and descending colons were the next 2.5 cm from the rectum.

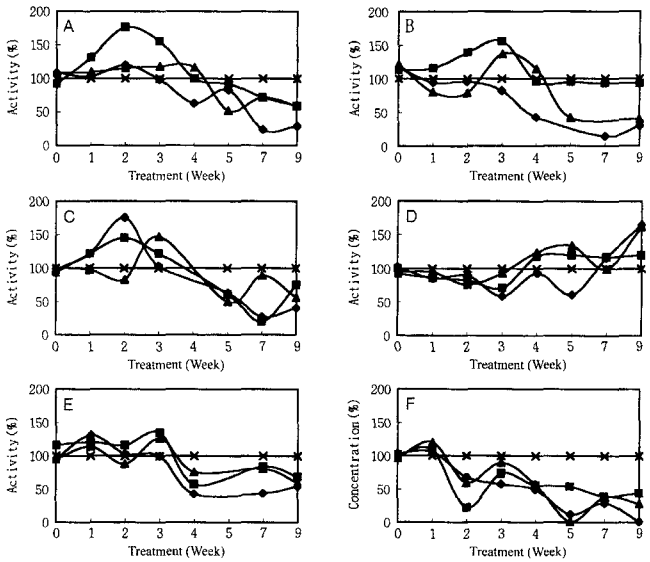

Fig. 1. *In vivo* inhibitory effect of the bifidobacteria isolated from Koreans for the harmful enzyme activities on mice and the production of ammonia from the fecal samples. During the experiment, the activities of fecal enzymes in normal mice varied, but their variations were not more than 30% of the mean value.

A, β-glucosidase; B, β-glucuronidase; C, tryptophanase; D, alkaline phosphatase; E, urease; F, ammonia concentration. ◆, K-110; ■, K-11; ▲, K-525; ×, control.

In Vivo Inhibitory Effect of *Bifidobacterium* spp. on Harmful Enzymes of Mouse Intestinal Microflora

The *in vivo* inhibitory effect of the bifidobacteria, which inhibited *in vitro* production of harmful enzymes of human intestinal microflora [6], on the harmful enzymes of mouse intestinal microflora, were investigated (Fig. 1). During the experiment, the activities of fecal enzymes in normal mice were varied, but not by more than 25% of the mean value. In particular, the fecal enzyme activities in mice were varied during 3 weeks after the administration of bifidobacteria, which might have been caused due to the change in experimental diets. However, the activities of fecal enzymes of the bifidobacteria-treated groups progressively decreased thereafter, compared to those of the control group. The activities of β-glucosidase, β-glucuronidase, tryptophanase, and urease were potently inhibited by the administration of bifidobacteria. The production of ammonia was also potently inhibited. However, alkaline phosphatase was not affected. Among the bifidobacteria tested, *B. breve* K-110 had the most potent inhibitory activity on the production of ammonia as well as fecal enzymes.

Preventive Effects of *Bifidobacterium* spp. on DMH-Induced Aberrant Crypt Foci Formation in Murine Colon

The preventive effects of bifidobacteria on DMH-induced aberrant crypt foci formation in the murine colon were

Table 1. Effect of the isolated bifidobacteria on DMH-induced aberrant crypt foci formation in the murine colon.

Treatment	Incidence	No. ACF/colon	No. AC/focus	Distribution (%)	
				R	S&D
Normal control	0/10	0	0		
DMH control	10/10	85.9±38.3[a]	5.3±0.90	23.5	76.5
DMH and *B. breve* K-110	9/10	7.2±5.7[b]	4.6±0.30	25.0	75.0
DMH and *B. breve* K-110	9/10	10.9±4.5[b]	4.7±1.69	26.2	73.8
DMH and *B. breve* K-110	9/10	6.6±3.4[b]	4.7±0.40	19.4	80.6

[a]Values are means±SD (n=10).
[b]Significantly different from DMH control ($p<0.05$).
R, rectum; S&D, sigmoidal and developing colon.

investigated (Table 1). DMH-induced ACF in mice were distinguished by their increased size, thickness of epithelial lining, and increased pericryptal zone (data not shown). The mice fed bifidobacteria and treated with normal saline showed no effect on ACF formation in the colon (Table 1). However, in mice fed normal diet with DMH treatment, an average of 68.9 ACF/colon was formed, whereas in mice administered *B. breve* K-110, *B. breve* K-111, and *B. infantis* K-525, the numbers of DMH-induced ACF reduced to 7.2, 10.9, and 6.6 ACF/colon, respectively. The mean number of crypts/focus was not significantly altered in the mice fed with the bifidobacteria compared to the group fed with *B. breve* K-110, *B. breve* K-111, or *B. infantis* K-525. Several previous observations suggest that the ACF are early preneoplastic lesions in the colon which are not present in the untreated laboratory animals but are induced by colon carcinogens. Our present results also indicate that *B. breve* K-110, *B. breve* K-111, and *B. infantis* K-525 inhibit DMH-induced ACF formation by 8.4%, 12.7%, and 7.7%, respectively. These results are significant because ACF is known to be the precursor lesions of chemically-induced colon cancer [5]. Inhibition of such precursor lesions by bifidobacteria in the current study suggests that these bifidobacteria might possess inhibitory activity against colon cancer.

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