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Inhibitory Effects of *Bifidobacterium* Spp. Isolated from a Healthy Korean on Harmful Enzymes of Human Intestinal Microflora

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Five hundreds of bifidobacteria were isolated from a healthy Korean and the inhibitory effects of these isloated bacteria on harmful enzymes of human intestinal microflora were examined by cocultivation of the isolated bifidobacteria with *E. coli* or total human intestinal microflora. In comparison with the results of *E. coli* or intestinal microflora cultivation, *Bifidobacterium breve* K-110, *B. breve* K-111 and *B. infantis* K-525 effectively inhibited harmful enzymes (β-glucuronidase and tryptophanase) of *E. coli* and lowered the pH of the culture media. Also they inhibited the harmful enzymes (β-glucosidase, β-glucuronidase, tryptophanase and urease) and ammonia production of intestinal microflora, and lowered pH of the culture media by increasing lactic acid bacteria of intestinal microflora. When these isolated bifidobacteria were administered on mice, fecal harmful enzymes were also inhibited. Among tested bifidobacteria, *B. breve* K-110 had the highest inhibitory effect of fecal harmful enzymes.

Key words : Bifidobacteria, Intestinal microflora, Harmful enzymes, β-glucuronidase, Tryptophanase

INTRODUCTION

Colorectal cancer is one of the most common forms of malignant tumor in human. Its incidence is high in United States, Canada, and Western Europe, but low in parts of Africans and Asia (Burkitt, 1975; Drasar and Irving, 1973). Recently, the death rate due to colon cancer in Korea has increased rapidly year by year. The rapid increase of colon cancer has been due to environmental changes, especially nutrition, rather than heredity (Berg and Hawell, 1974; Doll and Peto, 1981; Moore and Holdeman, 1975; Reddy and Wynder, 1973; Willet *et al.*, 1990; IARC, 1977). Higher fat intake increases the growth of colon bacteria capable of converting primary bile acids to carcinogenic substances as well as the induction of colon bacterial enzymes, β-glucuronidase, nitroreductase and tryptophanase, capable of converting procarinogen, such as amines and glucuronic acid conjugates of xenobiotics, to carcinogen (Burkitt, 1971; Fingold and Flora, 1975; Reddy *et al.*, 1992; Simon and Gorbach, 1984). From this relationship, colon bacteria may have an important role in colorectal cancer. In addition, the results of epidemiological studies supported this relationship between dietary fat and the risk of colon cancer (Reddy and Wynder, 1977).

Diets such as indigestible oligosaccharides and lactic acid bacteria are able to influence not only stool bulk but also intestinal bacterial metabolism (Barbolt and Abraham, 1980; Freeman *et al.*, 1980; Freudenheim *et al.*, 1990; Sugawara, 1993). The supply of lactic acid bacteria is an important factor for the growth of intestinal microflora as well as the inhibition of harmful enzymes produced by intestinal bacteria. The balance of these intestinal bacterial flora is closely related to human health conditions (Finegold-Suter *et al.*, 1977). Many kinds of lactic acid bacteria have been isolated from several sources, such as traditional foods and human stool, and their biological activities were measured (Salminen *et al.*, 1993; Tissier, 1900). Many researcher isolated *Bifidobacterium* spp. from human intestinal microflora, because bifidobacteria were normal major intestinal microflora of healthy human and their cell wall peptidoglycans were the immune stimulator (Mitsuoka, 1969; Scardi *et al.*, 1969). However, useful *Bifidobacterium* spp. were not isolated from normal intestinal microflora of healthy Koreans and inhibitory effects of lactic acid bacteria on harmful en-

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zymes of intestinal bacteria have not been reported. Here we isolated the beneficial *Bifidobacterium* spp. from normal intestinal flora and investigated inhibitory effect of the bifidobacteria on harmful enzymes of intestinal microflora.

MATERIALS AND METHODS

Materials

p-Nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-β-D-glucuronide, tryptophan, dimethyaminobenzaldehyde, urea, nitroprusside, fructose 6-phosphate, sodium thioglycolate, disodium p-nitrophenylphosphate hexahydrate and hydroxylamine were purchased from Sigma Chem. Co., (USA). The other reagents used were analytical grades. General anaerobic medium (GAM), Eggerth and Gagnon agar (E.G) and glucose blood liver agar (BL) were purchased from Nissui Pharm. Co, ltd., (Japan). Tryptic soy broth (TS) and agar were purchased from Difco, Co., (USA).

Isolation and identification of *Bifidobacterium* spp.

To isolate the bioactive *Bifidobacterium* spp. from healthy Korean man (in twenties, 60-70kg), the suspension of the fresh feces (1 g) of the healthy Korean man in GAM broth (9 ml) was diluted 10^5- to 10^8-fold with GAM broth. An aliquot (200 ul) of each diluted human feces was inoculated in EG, GAM, BL agar plate, which were anaerobically incubated at 37°C for 4 days. Each colony was subcultured in 10 ml of semisolid GAM media. This subcultured bacteria were cultured in GAM broth for the assay of the biochemical properties of *Bifidobacterium* spp. Among five hundreds of fructose 6-phosphate phosphoketolase (F 6PPK)-positive and gram positive bacteria, ten bioactive bifidobacteria were selected and then identification of the selected *Bifidobacterium* spp. was performed according to Bergey's mannual of systemic bacteriology (Scardi, 1986).

Cocultivation of bifidobacteria with *E. coli* HGU-3 or human intestinal microflora

Five Hundreds of *Bifidobacterium* spp. were cultured in GAM, respectively. 1×10^6 cultured bacteria of each *Bifodobacterium* spp. with 1×10^6 previously cultured *E. coli* HGU-3 were inoculated in 5 ml of GAM, anaerobically cultured for 20 h and centrifuged at 3,000 rpm for 10 min. Then the pH of resulting supernatant was measured. The precipitate was washed with cold saline and suspended in 0.1 M phosphate buffer (pH 7.0) and then the activities of harmful enzymes were measured. Also the activities of harmful enzymes were measured in different proportions of number of *Bifidobacterium* spp. to *E. coli* HGU-3 (10:1, 1:1, 1:10)

Enzyme activity assay (Gutmann and Bergmeyer, 1974; Kim *et al.*, 1992; Kim *et al.*, 1995)

β-Glucosidase activity was assayed as follows; 2 ml each reaction mixture consisting of 0.8 ml of 2 mM p-nitrophenyl-β-D-glucopyranoside and 0.2 ml of the enzyme solution (collected cells) was incubated for 30 min at 37°C and then stopped by addition 1 ml of 0.5 N NaOH. The stopped reaction mixture was centrifuged at 3,000 rpm for 10 min and measured the activity by monitering the absorbance at 405 nm.

β-Glucuronidase activity was assayed as follows; 2 ml each reaction mixture consisting of 0.8 ml of 2 mM p-nitrophenyl-β-D-glucuronide and 0.2 ml of enzyme solution (collected cells) was incubated for 30 min at 37°C and then stopped by addition 1 ml of 0.5 N NaOH. The stopped reaction mixture was centrifuged at 3,000 rpm for 10 min and was measured the activity by monitering the absorbance at 405 nm.

Tryptophanase was assayed as follows; the reaction mixture containing 0.2 ml of complete reaction mixture (2.75 mg pyrophosphate, 19.6 mg disodium EDTA dihydrate and 10 mg bovine serum albumin in 100 ml of 0.05 M potassium phosphate, pH 7.5), 0.2 ml of 20 mM tryptophan and 0.1 ml of enzyme solution (collected cells) was incubated for 1 h at 37°C. The reaction mixture was stopped by adding 2 ml of color reagent solution (14.7 g p-dimethylaminobenzaldehyde, 52 ml H_2SO_4 and 948 ml of 95% ethanol) and centrifuged at 3,000 rpm for 10 min. The enzyme activity was measured by monitering the absorbance at 550 nm.

Urease activity was assayed by the indophenol method with minor modification. Briefly, 100 μl of enzymes (collected cells) were added to 0.3 ml of urea substrate solution (4 mM urea in 20 mM phosphate buffer, pH 7.0) and incubated at 37°C for 30 min. The reaction mixture was terminated by 0.1 ml of 1 N H_2SO_4, added with phenolnitroprusside reagent and alkaline hypochlorite reagent, and incubated at 65°C for 20 min. The quantity of ammonia liberated from reaction mixture was determined from the standard curve correlating the absorbance at 630 nm.

Alkaline phosphatase activity was assayed as follows; the reaction mixture containing 0.2 ml of 2 mM disodium p-nitrophenylphosphate hexahydrate, 0.6 ml of NaOH glycine buffer (pH 8.5) and 0.2 ml of the enzyme solution was incubated for 15 min at 37°C and then 1 ml of 0.5 N NaOH was added. The stopped reaction mixture was centrifuged for 30 min at 37°C and the enzyme activity was measured by monitering the absorbance at 405 nm.

To meaure F6PPK, the cultured bacteria was washed twice with solution 1 (50 mM sodium phosphate buffer, pH 6.5, containing 0.05% cyteine HCl), suspended in solution 1 and sonicated (Heat system, USA). The sonicated cells was used as the crude en-

zyme. F6PPK activity was assayed as follows; the reaction mixture containing 0.25 ml of 6 mg/ml NaF containing 10 mg/ml sodium iodoacetate, 0.25 ml of 80 mg/ml fructose 6-phosphate and 1 ml of the enzyme solution was incubated for 30 min at 37°C and then added 1.5 ml of 139 mg/ml hydroxylamine HCl. In the stopped reaction mixture, 1 ml of 15% (w/v) trichloroacetic acid and 1 ml of 4 M HCl was added and then stood for 5 min. In the reaction mixture, 1 ml of 0.5% (w/v) $FeCl_3$ $6H_2O$ in 0.1 M HCl was added, centrifuged at 3,000 rpm for 10 min and the enzyme activity was measured by monitering the absorbance at 505 nm.

Animals

Fourty male mice (ICR 15 g) were purchased from Korea Food and Drug Administration. They were provided tap water and normal diet (Lab Chow, Samyang Co., ltd., Korea), housed at 23°C, 55±10% humidity for 10 days and used in experiment. They were randomly divided into 4 groups of ten animal each, and fed on different diets; The control group (Group 1) was fed with the normal diet (Samyang Co., ltd., Korea) only, Group 2 was fed with normal diet containing 1% K-110, which cultured in TS broth containing 0. 1% ascorbic acid and 0.01% sodium thioglycolate, Group 3 was fed with normal diet containing 1% K-111, which was cultured in TS broth containing 0.1% ascorbic acid and 0.01% sodium thioglycolate, Group 4 was fed with normal diet containing 1% K-525, which was cultured in TS broth containing 0.1% ascorbic acid and 0.01% sodium thioglycolate.

RESULTS AND DISCUSSION

Isolation of *Bifidobacterium* spp. inhibiting harmful enzymes of *E. coli*

More than five hundreds of bifidobacteria were isolated from intestinal bacteria of healthy Korean. These isolated bifidobacteria, which were gram-positive, F 6PPK-positive and anaerobic rod, were screened to have inhibitory effect on the harmful eznymes of intestinal *E. coli* HGU-3 and to lower intestinal pH by the production of organic acids, which could improve health conditions. Then, 200 bifidobacteria from more than five hundreds of bifidobacteria were selected. From these 200 *Bifidobacterium* spp., ten *Bifidobacterium* spp which had the potent inhibitory activity on harmful enzymes of *E. coli*, but did not show the activities of β-glucuronidase, tryptophanase and urease, were selected (Table I). Most of these selected *Bifidobacterium* spp. showed the potent F6PPK and β-glucosidase activity. However, K-309, K-311 and K-321 did not produce β-glucosidase. Also, these selected bacteria did not produce catalase, oxidase, indole, H_2S and nitroreductase. After the selected bifidobacteria were cocultured with *E. coli* HGU-3, the pH and the activity of harmful enzymes in the cocultured media were determined (Table II).

Compared to pH of the cultured medium of *E. coli* HGU-3, the cocultured media of K-103, K-105, K-110 or K-111 with *E. coli* HGU-3 had low final pH. Most of these selected bacteria inhibited tryptophanase productivity of *E. coli* HGU-3 except K-309 and β-glucuronidase productivity of *E. coli* HGU-3 except K-309, K-311, K-321 and K-513. After cocultivation of different ratio of bifidobacteria to *E. coli* HGU-3, the activity of harmful enzymes, β-glucuronidase and tryptophanase, were effectively inhibited by increasing the number of bifidobacteria on *E. coli* HGU-3. From these results, *Bifidobacterium* spp. inhibit not only harmful intestinal bacteria, but also harmful enzyme productivities of intestinal bacteria. Therefore, *Bifidobacterium* spp. should improve intestinal environment and decrease the risk of colon cancer.

Table I. pH and some enzyme activities of medium after culturing bifidobacteria isolated from human intestinal microflora

Bacterium	pH	Enzyme Activity[a]			
		F6PPK	β-Glucosidase	β-Glucuronidase	Tryptophanase
K-103	4.8	+	+++	-	-
K-105	4.6	++	+	-	-
K-110	4.8	+++	++	-	-
K-111	4.9	+++	+	-	-
K-309	5.5	+++	-	-	-
K-311	5.5	++	-	-	-
K-321	5.5	+++	-	-	-
K-506	4.8	+	+++	-	-
K-513	5.3	+	+++	-	-
K-525	5.4	+	++	-	-

[a]-: no, +: weak, ++: moderate, +++: strong enzyme activity

Table II. The effect of isolated bifidobacteria on the enzyme productivity of *E. coli* HGU-3, an human intestinal bacterium

Bacterium	pH	Inhibition[a]		
		β-Glucosidase	β-Glucuronidase	Tryptophanase
HGU-3	6.5			
K-103+HGU-3	5.0	-	+++	+++
K-105+HGU-3	5.1	-	+++	+++
K-110+HGU-3	5.1	+	+++	+++
K-111+HGU-3	5.3	+	+++	+++
K-309+HGU-3	6.2	++	++	++
K-311+HGU-3	6.2	+++	++	+++
K-321+HGU-3	6.2	+++	++	+++
K-506+HGU-3	5.2	-	+++	+++
K-513+HGU-3	5.8	-	+	+++
K-525+HGU-3	5.8	-	++	+++

[a]+: weak (<30% inhibition), ++: moderate (31-60% inhibition), +++: strong inhibition (61-100% inhibition).

In vitro inhibitory effect of the isolated *Bifidobacterium* spp. on the productivity of harmful emzymes of human intestinal microflora

After the ten selected bifidobacteria were cocultured with human intestinal microflora, final pH of the cocultured media and inhibitory effect of the bifidobacteria on the production of harmful enzymes and ammonia were measured (Fig. 1, Fig. 2). The productvity of ammonia and harmful enzymes, such as tryptophanase, β-glucuronidase and β-glucosidase, were inhibited by all selected bifidobacteria. Furthermore, the productivity of ammonia and harmful enzymes were effectively inhibited by increasing number of *Bifidobacterium* spp. Especially, the productivity of β-glucuronidase and tryptophanase were strongly inhibited. However, the productivity of alkaline phosphatase and urease were weakly inhibited by these bifidobacteria. When the ten selected *Bifidobacterium* spp. were cocultured with intestinal microflora, the effect of these

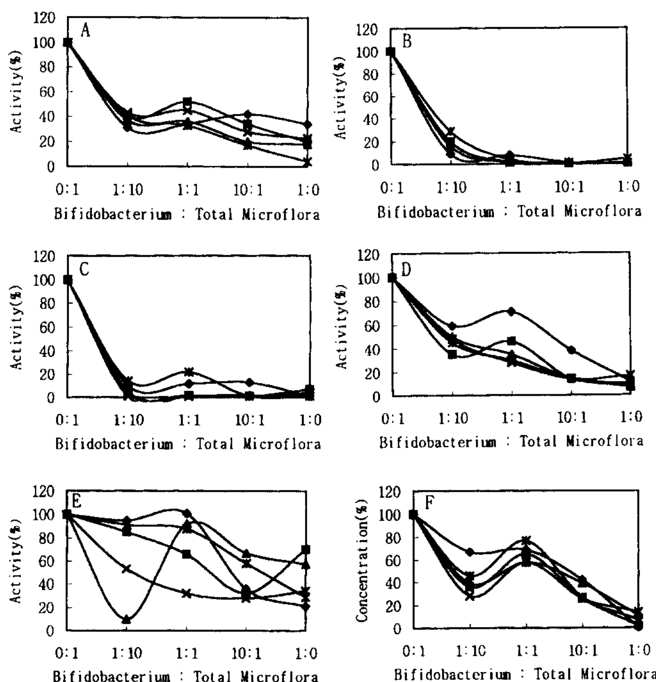

Fig. 1. The inhibitory effect of the isolated bacteria on harmfull enzymes activities and ammonia production of human intestinal microflora. A, β-glucosidase; B, β-glucuronidase; C, tryptophanase; D, alkaline phosphatase; E, urease; F, ammonia. ◆: K-103; ■: K-105, ▲: K-110. ×: K-111, ※: K-309.

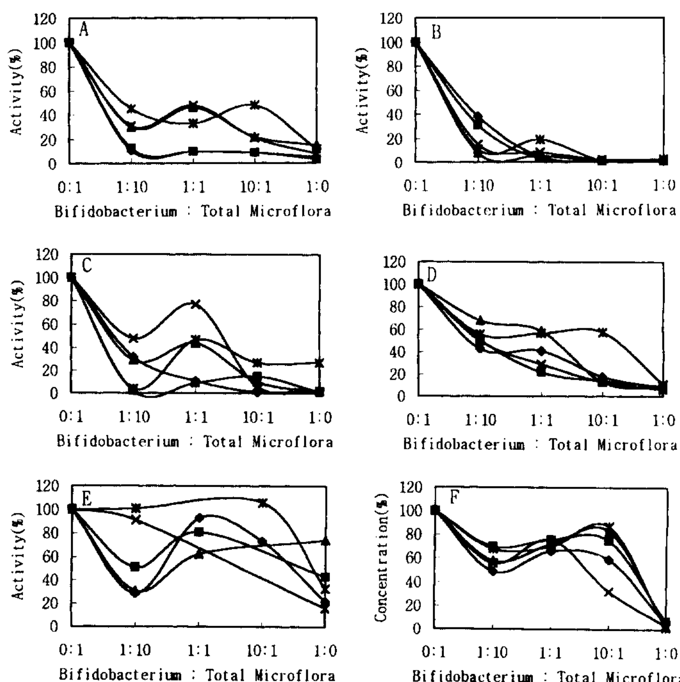

Fig. 2. The inhibitory effect of the isolated bacteria on harmfull enzymes activities and ammonia production of human intestinal microflora. A, β-glucosidase; B, β-glucuronidase; C, tryptophanase; D, alkaline phosphatase; E, urease; F, ammonia. ◆: K-311, ■: K-321, ▲: K-506, ×: K-513, ※: K-525.

Table III. Effect of isolated bifidobacteria on the bacterial composition of human intestinal microflora

Bifidobacterium	Ratio of Bifidobacterium/	# of cultured bacteria × 10^8 (A)	# of Bifidobacterium × 10^8 (B)	B/A × 100
K-103	10:1	19[a]	8	40
	1:10	263	41	16
K-105	10:1	223	10	4
	1:10	184	48	26
K-110	10:1	15	15	100
	1:10	410	40	10
K-111	10:1	7	6	86
	1:10	66	13	20
K-309	10:1	53	53	100
	1:10	93	39	42
K-311	10:1	368	368	100
	1:10	61	10	16
K-321	10:1	75	73	97
	1:10	104	27	26
K-506	10:1	97	67	69
	1:10	184	34	18
K-513	10:1	76	6	8
	1:10	144	77	53
K-525	10:1	15	6	40
	1:10	138	47	34
Total Microflora		230	62	27

The method empolyed for bacteriological analysis was basically identical to that reported by Mitsuoka *et al.* (1965).
[a]Human intestinal microflora with the isolated bifidobacteria were cocultured. The cocultured media were inoculated on BL agar plate and then cultured. After 3 days, number of bifidobacteria and total cultured bacteria were counted.

bifidobacteria on the bacterial composition of human intestinal microflora were determined (Table III). By increasing the ratio of each *Bifidobacterium* spp. on total intestinal bacteria. *Bifodobacterium* K-110, K-111, K-309 and K-321, except K-105 and K-513, increased selectively the number of *Bifidobacterium* spp. on the media cocultured with total intestinal bacteria. This increase in the number of bifidobacteria on the cocultured medium indicated that it could improve intestinal condition by the production of organic acid from bifidobacteria and reduce stool transit time, which reduces constipation and decrease the risk of colon cancer.

Identification of *Bifidobacterium* spp. isolated from a healthy Korean

After the isolated bifidobacteria, which could improve the intestinal environment, were incubated in the medium containing each carbohydrate, the utilization of each carbohydrate was determined by measuring pH and growth of bifidobacteria on the cultured media (Table IV). K-103 used most sugars, but did not use sorbitol and ribose. K-105 used lactose, but did not use sorbitol and raffinose. K-110 and K-111 used raffinose, ribose, starch and lactose, but did not use sorbitol and arabinose. K-309 used sorbitol and arabinose, but did not use starch. K-311 and K-321 used most of sugars, but did not use sorbitol and melezitose. K-506 used most of sugars, but did not sorbitol and lactose. K-513 used most of sugars, but did not use sorbitol and raffinose. K-525 used lactose, but did not use sorbitol and starch. On the basis of these results, K-103 was *Bifidobacterium cholerium,* K-105 was *B. bifidum,* K-110 was *B. breve,* K-111 was *B. breve,* K-309 was *B. catenulatum,* K-311 was *B. magnum,* K-321 was *B. magnum,* K-506 was *B. minimum,* K-513 was *B. cuniculi,* and K-525 was *B. infantis.*

In vivo inhibitory effect of the isolated *Bifidobacteirum* spp. on harmful enzymes of mouse intestinal microflora

The representative *Bifidobacterium* K-110, K-111 and K-525, which exhibited to improve intestinal environment among the 10 selected bacteria, were determined to *in vivo* inhibitory effect on harmful enzymes of mouse intestinal microflora. Four different diets (normal diet as control, normal diet containing 1% K-110, normal diet containing 1% K-111 and normal diet containing 1% K-525) were supplied to the mouse for 6 weeks. Then, the fresh feces from each diet group were collected every week. The activity of harmful enzymes of the collected feces were det-

Table IV. Utilization of carbohydrates of bifidobacteria selected from 500 isolated lactic acid bacteria of human

	Bifidobacterium K-103	*Bifidobacterium* K-105	*Bifidobacterium* K-110	*Bifidobacterium* K-111	*Bifidobacterium* K-309	*Bifidobacterium* K-311	*Bifidobacterium* K-321	*Bifidobacterium* K-506	*Bifidobacterium* K-513	*Bifidobacterium* K-525
Arabinose	-	-	+/-	+/-	+	+	+	+/-	+	+
Xylose	-	-	-	+/-	+	+	+	+/-	+	+
Ribose	+/-	+/-	+/-	+	+	+	+	+/-	-	+
Glucose	+/-	+/-	+/-	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+
Mannose	+/-	-	+	+/-	-	-	-	+	-	-
Fructose	-	+/-	+	+/-	+	+	+	+	+	-
Sucrose	-	+/-	+	+	+	+	+	+/-	+	-
Maltose	+/-	+	+	+	+	+	+	+	+	-
Cellobiose	-	-	-	+/-	-	-	-	+/-	-	-
Lactose	+	+	+	+	+	+	+	+/-	+	+
Trehalose	-	-	-	-	-	-	-	-	-	+
Melibiose	+	+	+	+	+	+	+	-	+	+
Raffinose	+	+	+	+	+	+	+	-	+	+
Melezitose	-	-	-	-	-	-	-	-	-	+/-
Dextrin	+	+	+	+	+	+/-	+/-	+	+/-	-
Starch	+	+	+	+	+	-	-	+	+/-	-
Glycogen	+/-	+	+	+	+	-	-	+	+/-	-
Inulin	-	+/-	-	+/-	+/-	-	-	+/-	-	-
Mannitol	-	+	+	+	+	-	-	+	-	+
Sorbitol	+/-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	+
Esculin	-	-	-	+/-	-	-	-	-	-	+
Salicin	+	+	+	+	+	-	-	+	-	+
Amygdalin	-	-	-	+/-	+/-	-	-	-	-	-
Gluconate	-	-	-	+/-	-	-	-	-	-	-
Shape	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod
Gram stain	+	+	+	+	+	+	+	+	+	+
Oxygen	anaerobe	anaerobe	anaerobe	anaerobe	anaerobe	anaerobe	anaerobe	anaerobe	anaerobe	anaerobe
F6PPK	+	+	+	+	+	+	+	+	+	+
	B. cholerium[a]	*B.bifidum*	*B.breve*	*B.breve*	*B.catenulatum*	*B.magnum*	*B.magnum*	*B.minimum*	*B.cuniculi*	*B.infantis*

[a]The scientific name was identified according to Bergey's manual.

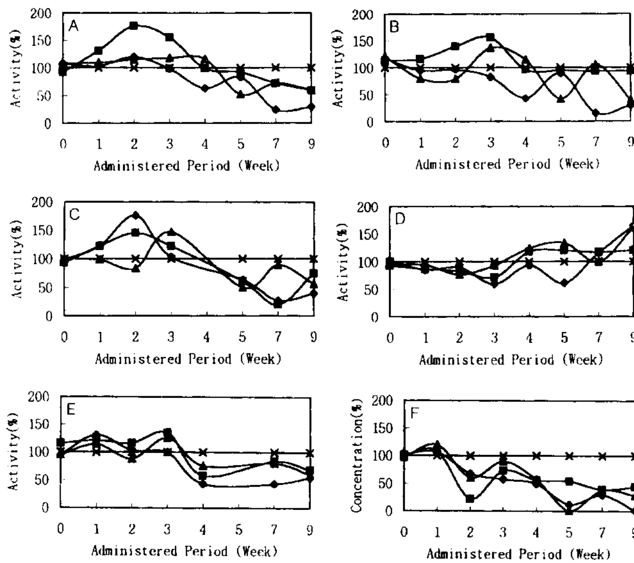

Fig. 3. *In vivo* inhibitory effect of the isolated bacteria on harmfull enzymes activities and ammonia production of the feces on mice. A, β-glucosidase; B, β-glucuronidase; C, tryptophanase; D, alkaline phosphatase; E, urease; F, ammonia. ◆: K-110, ■: K-111, ▲: K-525, ×: control.

ermined (Fig. 3). The inhibitory effects of diets containing each *Bifidobacterium* spp. (K-110, K-111 and K-525) on the fecal β-glucosidase activity of the mice were not observed during 3 weeks of feeding. However, compared to the fecal β-glucosidase activity of the control, the fecal β-glucosidase activity of K-110 treated group was strongly inhibited after 4 weeks of feeding the diet. The fecal β-glucosidase activities of K-111 or K-525 treated group were weakly inhibited after 5 week of feeding. The inhibitory effects of diets containing each *Bifidobacterium* spp. (K-110, K-111 and K-525) on the fecal β-glucuronidase activity of the mice were not observed during 3 weeks of feeding. However, compared to the fecal β-glucuronidase activity of the control, the fecal β-glucuronidase activity of K-110 treated group was strongly inhibited after 4 weeks of feeding. The fecal β-glucuronidase activities of the other group were weakly inhibited after 5 weeks of feeding. The inhibitory effects of diets containing each *Bifidobacterium* spp. (K-110, K-111 or K-525) on the fecal tryptophanase activitiy of the mice were not observed during 4 weeks of feeding. However, compared to the tryptophanase activity of the control, the fecal tryptophanase activities of all bifidobacteria treated groups were strongly inhibited after 5 weeks of feeding. Among the three groups, the group fed K-110 had the lowest tryptophanase activity of the feces. The inhibitory effects of diets containing each *Bifidobacterium* spp. (K-110, K-111 or K-525) on the fecal urease activity of the mice were not observed during 3 weeks of feeding. However, compared to the fecal urease activity of the control, the fecal urease activity of all bifidobacteria treated groups were strongly inhibited after 4 weeks of feeding. Among the three groups, the group fed K-110 had the lowest pH of the feces. Futhermore, after 2 weeks of feeding diets containing each *Bifidobacterium* spp., the strong inhibitory effects on the fecal ammonia produciton were appeared compared to that of the control. However, during feeding diets containing K-110, K-111 or K-525, the inhibitory effects on the fecal alkaline phosphatase activity of the mice were not observed. The fecal pHs of each group were measured (data not shown). The fecal pH of the mouse fed K-110, K-111 or K-525 with normal diet showed low pH comparing to the fecal pH of the control. Among the three groups, the group fed K-110 had the lowest pH of the feces.

When the three selected *Bifidobacterium* spp. were administered on mice, the effects of these bifidobacteria on the bacterial composition of mouse intestinal microflora were determined (Table V). These three selected bifidobacteria increased selectively intestinal bifidobacteria of mice. This increase in the number of bifidobacteria on mouse intestine indicated that it could improve intestinal environment and reduce stool transit time, which reduces constipation and decrease the risk of colon cancer.

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Table V. Effect of Korean bifidobacteria on mouse intestinal microflora

Bifidobacterium[a]	# of Bifidobacteria $\times 10^8$	# of Bacteroides $\times 10^8$	# of Eubacterium $\times 10^8$	% of Bifidobacteria[b]
K-110	39	38	18	41
K-111	39	36	7	48
K-525	48	10	48	45
Control	28	36	23	32

The method empolyed for bacteriological analysis was basically identical to that reported by Mitsuoka *et al.* (1965).
[a]orally administered 6 g/kg of isolated *Bifidobacterium* spp. with diet.
[b]number of bifidobacteria per number of bacteria cultured on BL plate.

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