Effect of *Panax ginseng* Extract on Growth Responses of Human Intestinal Bacteria and Bacterial Metabolism

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Abstract □ The growth responses of a variety of human intestinal bacteria to extracts of *Panax ginseng* and five other oriental medicinal Araliaceae were evaluated *in vitro* and *in vivo*. The extracts enhanced the growth of *Bifidobacterium breve* and *B. longum* in media with or without carbon sources, suggesting that bifidus factor(s) might be involved in the phenomenon. This effect was most pronounced with water extract of *P. ginseng*, the growth of 27 bifidobacteria strains belonging to *B. adolescentis, B. longum, B. breve* and *B. infantis* being greatly stimurated, whereas seven *B. bifidum* strains and other bacteria such as clostridia and *Escherichia coli* had little or no ability to utilise it for growth. Methanol extracts of *P. ginseng* were found to selectively inhibit growth of various clostridia including *C. perfringens* and *C. paraputrificum*, but this effect was not observed on other bacteria including bifidobacteria.

The effect of ginseng extract intake (600 mg/day for two weeks) on the faecal microflora, pH, volatile fatty acids, ammonia, putrefactive products, and -glucuronidase, -glucosidase and nitroreductase activities, and on the blood components (triglyceride, total cholesterol and ammonia) were investigated using seven healthy human volunteers. The total concentration of faecal microflora including *Bifidobacterium* spp. during the period of ginseng extract intake was significantly unaffected from the preceding and subsequent control peroids. However, the frequency of occurrence of subjects having *C. perfringens* was significantly decreased. The faecal pH value was also significantly decreased, suggesting that the intake might increase the activity of *Bifidobacterium* spp. Other biochemical properties in faeces did not changed significantly. The levels of ammonia and triglycerid in blood were decreased with ginseng extract intake.

These results may be an indication of at least one of the pharmacological actions of *P. ginseng* as an adaptogen.

Keywords □ Araliaceae plant, *Panax ginseng*, intestinal bacteria, bifidus factor(s), growth inhibition, faecal microflora, faecal enzyme activity, volatile fatty acids, putrefactive products, blood components

Introduction

Various kinds of microorganisms are resident in the human intestinal tract as a highly compelx ecosystem with considerable species diversity. It is well known that they not only participate in normal physiological functions, but also contribute significantly to the genesis of various disease states by biotransforming a variety of ingested of endogenously formed compounds to useful of harmful derivatives. Accordingly, this biotranformation may influence drug efficacy, toxicity, carcinogenesis and ageing.

The term 'adaptogen' is defined as a substance that increases non-specific resistance of the organisms to environmental stress and disease. Adapto-

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genic activity was first reported for *Eleutherococcus senticosus*.[6] Evidence has ben accumulating that *Panax ginseng* also contains adaptogens.[6,22,24] These two plants belong to the family Araliaceae. However, the effect of extracts form Araliaceae plants on the human intestinal microorganisms remains unknown, although much current concern is focused on these organisms in relation to human health. We have therefore examined the effect of *Panax ginseng* extract on a variety of intestinal bacteria and bacterial metabolism.

Materials and Methods

In vitro experiments

Bacteria and culture conditions

The bacterial strains used in this study were as follows; 27 bifidobacteria, 19 bacteroides, 15 clostridia, 15 eubacteria, eight lactobacilli, five *E. coli*, five peptostreptococci, four mitsuokellae, three fusobacteria, two propionibacteria, and one each of *Streptococcus, Megasphaera, Rikenella* and *Megamonas* species. They were, except as noted, from the RIKEN culture collection. Stock cultures of all strains were routinely stored on EGLF agar at −80 °C and when required were subcultured on BL and EG agar (Eiken Chemical Co., Ltd, Tokyo, Japan) with 5 per cent horse blood for bifidobacteria and other organisms, respectively. All plates were incubated for 2d at 37 °C in an atmosphere of 100 per cent CO_2. On the following day, bifidobacteria were grown in Briggs liver broth (pH 6.8) in an atmosphere of 100 per cent CO_2, whereas other kinds of bacteria were grown in EGF broth (pH 7.2) in an atmosphere of 80 per cent N_2, 15 per cent CO_2 and 5 per cent H_2. All cultures were checked for contamination at the end of the growth cycle.

Plant materials and sample preparation

The medicinal Araliaceae plants used in this study were as follows: four-year-old *P. ginseng* (root); *P. japonicum* (root); *P. notoginseng* (root); *Acanthopanax* sp. (cortex); *Aralia elata* (cortex); *A. cordata* (root). These samples were finely powdered using a blender, extracted three times with methanol at 25 °C and filtered (Toyo filter No. 2). The combined filtrate was concentrated *in vacuo* at 35 °C. The residue was extracted with water at 80 °C for 1 h and filtered. The filtrate volume was reduced with a rotary evaporator and freeze dried *in vacuo*.

Micorbiological assay

For growth measurements with microorganisms, the testing methods of Mitsuoka[27] were applied. In the experiments for bifidus factor(s) derived from non-carbon sources, György broth[15] (pH 6.8) as modified by Yoshioka[39] was used. In the experiments for bifidus factor(s) derived from carbon sources, PYF broth (pH 7.8) was used. Bacteria grown in Briggs liver broth or EGF broth were centrifuged at 3000 r.p.m. for 10 min, washed three times with 10 m*l* of sterile physiological saline (0.85 per cent NaCl, 0.1 per cent L-cysteine-HCl, and 0.1 per cent sodium thioglycolate), and suspended in 5 m*l* of reduced saline. Two drops of the suspension were inoculated on to the media described above. Filter-sterilised test materials and ascorbic acid solution (sterilised at 115 °C for 20 min) were added to the media in a final volume of 10 m*l*. Solution of the test materials were prepared using methanol of distilled water as a solvent. The methanol concentration in the solutions did not exceed 2 per cent which was found to be without adverse effect on the bactera tested. Samples from test and control solutions were assayed by the membrane filter procedure. The media were incubated anaerobically at 37 °C for 48 h, and the bacterial growth determined by change in pH value.

The growth response to the test samples was determined by comparing with the value of each control. The responses were classified as follows: the strongest response + +2, pH 4.5-5.0 for modified György broth and PYF broth; moderate +, pH 5.1-5.5 for the same broths; weak (+), pH 5.6-6.0 for PYF broth; and no response, −. Each assay was repeated three or more times.

For assay of the inhibitory effect of *P. ginseng* on the organisms, one loopful of bacteria was suspended in 1 m*l* of sterile physiological saline. An aliquot

(0.1 m*l*) of the bacterial suspensions was seeded on Brucella agar (Difco) supplemented with 5 per cent horse blood. A sample (10 mg) in methanol or water solution (100 u*l*) was applied by Drummond glass microcapillary to a paper disc (ADVANTEC 8 mm Toyo Roshi, Japan). After evaporation of solvents, the paper discs were incubated for 2 d at 37 °C in an atmosphere of 80 per cent N_2, 15 per cent CO_2 and 5 per cent H_2. Control discs received methanol or water only. All tests of inhibition were performed at least in duplicate, and a mean inhibition zone of 10 mm of greater was considered positive (+).

In vivo experiments

Subjects and diets

Seven healthy volunteers (laboratory staffs at RIKEN); four males and three females between 25 and 37 years old) had the Japanese-style diets. None of the subjects had been on antibiotic treatment or other therapy for six months prior to collection of the faecal samples. They were served 200 mg ginseng extract after each usual diet. Total consumption of the extract was 600 mg per day. The duration of the intake was two weeks. Finally they were given no ginseng extract.

Ginseng sample preparation

Four-year-old ginseng roots were used. They were finely powdered using blender, extracted three times with 50% ethanol and filtered. The combined filtrate was concentrated *in vacuo* at 35 °C and freeze dried *in vacuo*.

Table 1. Culture media and methods

Medium	Main enumerated microorganisms	Diluted to be plated[a]	Incubation time (days)
Aerobic incubation:			
TS blood agar	Predominant aerobes	$10^{-5, -6, -7}$	1
DHL agar	Enterobacteriaeae		
TATAC agar	*Streptococcus*	$10^{-1, -3, -5, -7}$	2
PEES agar	*Staphytococcus*		2
P agar	Yeasts and molds		2
Anaerobic incubation[b]			
M10 agar	Predominant anaerobes	$10^{-7, -8}$	3
EG agar	Predominant anaerobes	$10^{-6, -7, -8}$	2
BL agar	Predominant anaerobes		2
NBGT agar	Bacteroidaceae		2
BS agar	*Bifidobacterium*		2
ES agar	*Eubacterium*	$10^{-1, -3, -5, -7}$	2
VS agar	*Veillonella and Megasphaera*		2
LBS agar	*Lactobacillus*		2
NN agar	*Clostridium perfringens*		2

[a] An aliquot (0.05 m*l*) of each dilution was plated on each medium used.

[b] M10 agar was prepared by using the "plate-in-bottle" method, and other media by a steelwool-jar filled with 100% CO_2. TS, trypticase soy (BBL); DHL, deoxycholate-hydrogen sulfide-lactose; TATAC, triphenylaterazoliumazide-thallous sulfate-acridine arange-crystal violet; PEES, phenylethyl alcohol-egg yolk-Staphylococcus no. 110 (EIKEN); P, potato dextrose; M10, medium 10; Eg, Eggerth Gagnon; BL, glucose-blood-liver; NBGT, nemycin-brilliantgreen-taurocholate-bolld; BS, *Bifidobacterium* selective; ES, *Eubacterium* selective; VS, *Veillonella* selective; LBS, *Lactobacillus* selective (BBL); NN, neomycin Nagler.

Faecal sample preparation and assays

Fresh faecal specimens were collected from each subject on day 10 and 14 in the course of each period. Faecal microflora were analysed by the method of Mitsuoka *et al.*[31,32], the culture media and method for isolation and identification being shown in Table 1. Bacterial colonies grown on each medium were counted and identified according to the colonial and cellular morphologies, Gram stain, spore formation, and aerobic growth. The bacterial count per gram of wet faeces was calculated and converted into a logarithmic equivalent. The total viable count was calculated from the sum of the counts of each bacterial species.

Chemical and biochemical properties of faeces

The faecal pH values were measured directly by inserting a glass-electrode. Volatile fatty acids (acetate butylate, and propionate), putrefactive products (indole, cresol, and others), and ammonia were measured.[33] A Shimadzu GC 9A gas chromatograph (Shimadzu, Japan) with a flame ionization detector fitted with Unisole F-200 (3.2 mmID × 2.1 m) (Gaschrom Kogyo Japan). The column conditioned at 180 °C for 2h before use. The column temperature was 120 °C (isothermal); the injection port and detector temperature was 200 °C. Nitrogen gas at a column flow rate of 32 m*l*/min was used as the carrier gas. Bacterial m-glucuronidase (substrate: p-nitrophenyl-m-D-glucuronide), m-glucosidase (p-nitrophenyl-m-glucopyranodise), and nitroreductase (p-nitrobenzoic acid) activities were determined under anaerobic conditions as described previously.

Blood compounds

Total cholesterol, triglyceride, and ammonia in blood were measured as described previously.

Results

The effects of extracts from six Araliaceae plants on the growth of bifidobacteria are given in Table 2. For determination of bacterial growth, two kinds of media were used: modified György broth as a carbon source-containing medium and PYF broth as a carbon source-free medium. *Bifidobacterium longum* and *B. breve* were used as representatives of the organisms dominant in intestines of adults and infants, respectively. In modified György broth, all water extracts from the plants tested showed either strong or moderate growth-promoting activity for both *B. longum* and *B. breve*. Methanol extracts from all except *A. elata* showed little or no growth stimulation on *B. longum*. However, the methanol extracts from *P. notoginseng*, *Acanthopanax* sp. and *A. elata* stimulated growth of *B. breve*.

Table 2. Effect of extracts from Araliaceae plants on the growth of *B. longun* and *B. breve*

Test plant	B. longum E194b		B. breve S1	
	Gyorgy	PYF	Gyorgy	PYF
Panax ginseng				
M	−	−	−	++
W	++	++	++	++
P. japonicum				
M	−	−	−	−
W	++	+	++	++
P. notoginseng				
M	−	−	++	++
W	++	−	++	++
Acanthopanax sp.				
M	−	+	++	++
W	++	+	++	++
Aralia elata				
M	++	++	++	++
W	++	+	++	+
A. cordata				
M	−	−	−	−
W	++	(+)	++	(+)

Results obtained in the test on PYF broth were similar although the quantitative response was slightly different (Table 2).

Dose-growth responses for five bifidobacteria strains were studied using extract from 4-y-old. *P. ginseng* root (Table 3). Water extract of *P. ginseng* (PWE) at 1 per cent (w/v) strongly enhanced the growth of *B. adolescentis*, *B. longum*, *B. breve* and *B.*

infantis in both procedures. Moderate responses were obtained at 0.1 per cent (w/v) PWE on György broth, but this concentration produced no growth response on PYF broth. No effect on bacterial growth in either broth was seen with 0.01 per cent PWE. The methanol extract of *P. ginseng* (PME) showed little or no growth-promoting effect on the five bifidobacteria tested.

The bifidus factors so far reported are known to be effective on only some strains of bifidobacteria. Therefore, the effect of PWE on a variety of bifidobacteria were investigated (Table 4). The result obtained by using 1 per cent PWE showed that growth response was indeed strain dependent PWE enhanced the growth of many strains of *B. adolescentis*, *B. longum*, *B. breve* and *B. infantis* strongly or moderately in the test on both media described above, whereas none was observed with six *B. bifidum* strains.

Table 5 shows the effect of 1 per cent PWE on various kinds of other microorganisms. The effect was also strain dependent. PWE showed strong growth-promoting activity for lactobacilli on either György or PYF broth. However, other microorganisms including eubacteria, clostridia and *E. coli* showed little or no growth in the two media with 1 per cent PWE.

Inhibitory effects of PME and PWE on the bacteria mentioned above were also examined by paper disc method (Table 6). At a concentration of 10

Table 3. Bifidobacteria gorwth-promoting activity at various concentrations of P. ginseng*

Strain	György						PYF					
	PWE			PME			PWE			PME		
	0.01	0.1	1	0.01	0.1	1	0.01	0.1	1	0.01	0.1	1
B. adolescentis E194a	−	++	++	−	−	+	−	−	++	−	−	++
B. longum E194b	−	++	++	−	−	−	−	−	++	−	−	−
B. breve S1	−	+	++	−	−	−	−	(+)	++	−	−	++
B. infantis S12	−	+	++	−	−	−	−	−	++	−	−	−
B. bifidum Ti	−	−	−	−	−	−	−	−	−	−	−	−

PWE = water extract (w/v, %) of *P. ginseng*; PME = methanol extract (w/v, %) of *P. ginseng*.
*Four-year-old ginseng root was used.
+György broth[15] modified by Yoshika[39].

Table 4. Growth responses of many srains of bifidobacteria to PWE*

Strain	Growth response	
	György+	PYF
B. adolescentis E-298b	+	(+)
E-319a, M-101-4, M-602 and U-601	++	++
M-601, S-601 and S-602	++	−
B. longum Kd-5-6, M-101-2 and S-601	++	−
M-601 and S-3	+	−
B. breve I-53-8w and S-46	++	++
B. infantis I-10-5	++	−
B. bifidum A-234-4, E-319, M-601, S-28a, S-601 and S-602	−	−

Responses were scored as described in the text.
*Water extract of *P. ginseng*.
+György broth[15] modified by Yoshioka[39].

Table 5. Growth responses of various strains of intestinal bacteria except bifidobacteria to PWE*

Strain	Growth response	
	György+	PYF
Lactobacillus casei ATCC-7469	++	−
IFO-3425	−	−
L. acidophilus ATCC-4356 and Omfl	−	++
L. gasseri F-164	−	++
M-601	++	++
L. salivarius ATCC-11741 and ATTC-11742	−	++
Bacteroides distasonis B-26, M-602, M-603, S-601 and U-604	−	+
B. fragilis 3676 and M-601	−	+
B. melaninogenicus NCTC-9337	−	−
B. thetaiotaomicron AS-126	−	+
B. uniformis M-601	−	+
B. vulgatus B-19 and B-24	−	++
S-601, 602, 603, 604, 606 and F-92	−	+
S-605	−	−
Clostridium bifermentans B-1 and B-4	−	−
C. butyricum ATCC-14823	−	++
S-601	−	−
C. coccoides B-2	−	(+)
C. difficile ATCC-9689	−	−
C. innocuum M-601	−	−
C. paraputrificum B-3-4, B-78 and VPI-6372	−	−
C. perfringens ATCC-13124, C-01 and B-165-16	−	−
C. ramosum ATCC-25582 and C-00	++	++
Eubacterium aerofaciens M-608, M-609, M-610, S-601, S-602, S-604, S-605 and S-606	−	−
E. lentum M-601	−	−
E. limosum ATCC-8486 and E-1	−	−
VPI-1939	−	+
E. nitritogenes ATCC-25547	+	+
E. tortuosum ATCC-25548	−	+
Escherichia coli E-605	−	+
M-602 and O-601	−	−
Mitsuokella multiacida F1-376	+	++
NCTC-10934, NCTC-10935 and P-208-58	++	++
Peptostreptococcus anaerobius X-36	−	−
P. asaccharolyticus VPI-5045A	−	−
P. parvulus 1612	−	−
P. prevotii ATCC-9321	−	−
P. productus ATCC-27340	−	+
Streptococcus faecalis IFO-3971	−	++
Propionibacterium acnes ATCC-6919 and ATCC-11829	−	−

mg/disc, PME inhibited various strains of clostridia including *C. perfringens* and *C. paraputrificum*. PWE showed no inhibitory effect on these bacteria by this method.

The changes of faecal microflora and frequency of occurrence [(number of subjects with organisms detected/number of subjects examined) × 100] by ginseng extract intake are given in Table 7. Al-

Table 5. Continued

Strain	Growth response	
	György+	PYF
Fusobacterium biacutus PAS-4476	+	(+)
F. necrophorum W-12, 2013	–	–
F. varium P103-112	–	–
Megasphaera eisdenii F1-375	–	–
Rikenella microfusus NCTC-11190	–	(+)

Responses were scored as described in the text.
*Water extract of *P. ginseng*.
+ György broth[15] modified by Yoshioka[39].

Table 6. Inhibitory effects of *P.ginseng* on various intestinal bacteria

Strain	Growth inhibition*	
	PME	PWE
Bifidobacterium adolescentis E-194a	–	–
B. longum E194b	–	–
B. breve S1	–	–
B. infantis S12	–	–
B. bifidum Ti	–	–
Bacteroides distasonis M-602 and S-601	–	–
B. fragilis 3676 and M-601	–	–
B. thetaiotaomicrom 6AS-126	–	–
B. vulgatus F-92 and S-601	–	–
Clostridium bifermentans B1	–	–
C. butyricum ATCC-14823 and S-601	+	–
C. coccoides B-2	+	–
C. difficile ATCC-9689	– –	
C. innocuum M-601	–	–
C. paraputrificum B-78 and VPI-6372	+	–
C. perfringens ATCC-13124,	–	–
C-01 and B-165-16	+	–
C. ramosum ATCC-25582 and C-00	+	–
Eubacterium aerofaciens S-601 and S-605	–	–
S-605	+	–
Escherichia coli E-605, F-604, M-602, O-601 and V-603	–	–

PME = methanol extract of 4-year-old *P. ginseng root (10 mg/disc);*
PWE = water extract of 4-year-old *P. ginseng* root (10 mg/disc).
*Inhibition zone of 10 mm or greater was considered positive (+).

though some variation in individuals, in general, the concentrations of faecal bacteria between controls and treats were comparble. The total mean concentration of the faecal bacterial genera per g wet faeces during the period of ginseng extract intake did not differ significantly from the preceding and subsequent control periods. There was also no difference in the mean total microscopic count of the faecal flora by ginseng extract intake. However, the frequency of occurrence of subject having *C. perfringens* during the period of ginseng intake were significantly lower from the preceding and subsequent control periods. At the 2nd week after discontinuance of ginseng extract intake, the frequency of occurrence seemed to appear to return to the preceding control period.

Effect of ginseng extract intake on faecal pH was investigated (Fig. 1), because it has been well known that the development of bifidus flora significantly caused lower pH levels of faceces. The faecal pH values during the period of ginseng extract intake were lower from the preceding and subsequent control periods. Discontinuance of the intake caused the increase in pH values of all subjects.

The interaction between ginseng and faecal bacterial activity was investigated, because many of the bacterial enzymes play large roles in human health (Fig, 2). During initial experiments it was observed that the seven individuals were very variable in the activities of the faecal bacterial enzymes measured. Although this variation may be attributable to the differences in faecal water or bacterial content, biochemically important changes in enzyme in the aqueous phase of the faeces may be presented as per g wet weight. Faecal m-glucuronidase and m-glucosidase activities per g wet faeces slightly increased, but nitroreductase activity tended to decrease with ginseng extract intake. These differences, however, were not statistically significant.

Table 7. Effect of ginseng extract itake on the human flora

	CONT A-1	CONT A-2	TEST 1	TEST 2	CONT B-1	CONT B-2
Total counts	10.6±0.1	10.7±0.1	10.8±0.2	10.6±0.3	10.7±0.2	10.8±0.2
Bacteroidcene	10.3±0.2 (7/7)[b]	10.5±0.1 (7/7)	10.4±0.3 (7/7)	10.3±0.3 (7/7)	10.4±0.2 (7/7)	10.4±0.2 (7/7)
Bubacterius	9.9±0.2 (7/7)	10.1±0.3 (7/7)	10.1±0.2 (7/7)	9.9±0.3 (7/7)	10.1±0.2 (7/7)	10.0±0.3 (7/7)
Peptococcuceae	8.7±1.6 (6/7)	9.1±0.4 (7/7)	9.1±0.6 (7/7)	9.1±0.4 (5/7)	9.3±0.5 (5/7)	9.8±0.2 (4/7)
Bifidobacterium	9.8±0.5 (7/7)	9.8±0.3 (7/7)	10.0±0.3 (7/7)	9.7±0.5 (7/7)	10.1±0.3 (7/7)	9.9±0.2 (7/7)
Veillonella	5.8±1.6 (6/7)	6.6±1.2 (5/7)	4.5±1.6 (6/7)	5.9±1.0 (6/7)	4.5±0.8 (5/7)	5.5±1.3 (4/7)
Megasphaera	6.6 (1/7)	6.1 (1/7)	6.3 (1/7)	6.1 (1/7)	6.3 (1/7)	(0/7)
Curved rods	(0/7)	(0/7)	(0/7)	(0/7)	(0/7)	(0/7)
CL. perfringens	3.7±1.2 (5/7)	4.3±1.4 (4/7)	6.1±1.3 (2/7)	5.3±0.4 (2/7)	(0/7)	5.0 (1/7)
Clostridium-other	7.6±0.9 (7/7)	8.0±0.5 (7/7)	7.7±1.2 (7/7)	8.0±0.5 (7/7)	8.0±0.5 (7/7)	8.0±0.9 (7/7)
Lactobacillus	5.7±1.4 (7/7)	6.4±1.5 (7/7)	6.6±1.2 (7/7)	5.7±1.2 (7/7)	5.7±1.0 (7/7)	5.3±1.3 (7/7)
Enterobacteriaceae	7.3±1.1 (7/7)	7.2±1.1 (7/7)	7.3±1.3 (7/7)	7.3±1.0 (7/7)	7.1±1.2 (7/7)	7.7±1.4 (7/7)
Streptococcuceae	7.1±1.8 (7/7)	7.0±1.4 (7/7)	7.2±1.1 (7/7)	7.3±1.0 (7/7)	6.2±1.3 (7/7)	6.8±1.0 (7/7)
Micrococcucene	3.3±0.9 (4/7)	2.8±0.6 (4/7)	2.9±0.3 (6/7)	3.0±0.5 (4/7)	3.0±0.7 (4/7)	3.0±0.5 (7/7)
Bacillus	6.3 (1/7)	2.5±0.1 (3/7)	(0/7)	3.0±0.1 (2/7)	3.1 (1/7)	2.9 (1/7)
P. aeruginosa	3.3±0.3 (3/7)	2.7±0.8 (4/7)	3.3±1.1 (4/7)	3.3±1.4 (4/7)	2.6±0.2 (3/7)	3.5±1.2 (2/7)
Corynebacterium	5.8 (1/7)	4.3±0.0 (2/7)	3.7±0.9 (4/7)	5.3 (1/7)	4.0 (1/7)	3.7±1.0 (3/7)
Yeasts	1.8±2.1 (3/7)	3.0±0.4 (3/7)	2.7±0.3 (3/7)	2.8±0.5 (4/7)	2.8±0.5 (4/7)	3.3±1.2 (3/7)
Moids	(0/7)	(0/7)	(0/7)	(0/7)	(0/7)	(0/7)

[a] Mean S.D. of log no. of bacteria per gram of wet faeces

[b] Frequency of occurrence [(number of subjects with organism detected/number of subjects examined)×100]

Effect of ginseng extract intake on faecal metabolites are shown in Fig. 3. The period of the intake was without any significant effect on putrefactive products (indole, *p*-cresole and others), volatile fatty acids (acetate, butylate, propionate and others), and ammonia in faeces.

Blood components were affected by the ginseng extract intake (Fig. 4). The concentrations of triglyceride and ammonia in blood were decreased with the intake, although total cholesterol concentration was not significantly decreased.

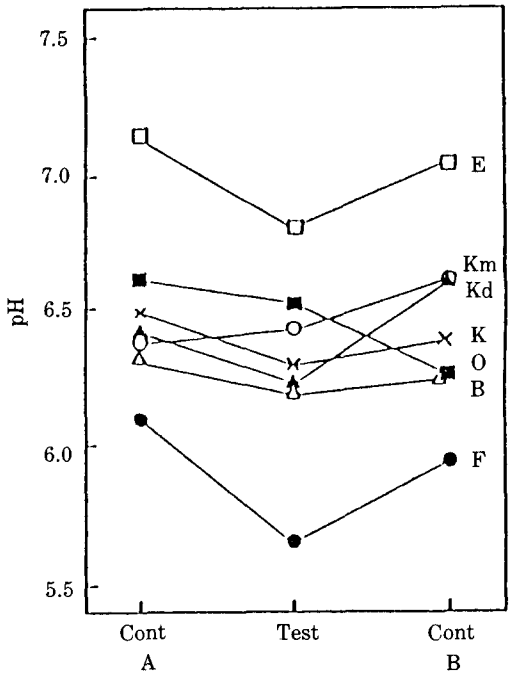

Fig. 1. Effect of ginseng extract intake on faecal pH. The mean faecal pH values in Con A, Test and Con B were 6.42, 6.30 and 6.44, respectively.

Discussion

The intestinal microflora in healthy man remains relatively constant but is known to be greatly influenced by physical, biological, chemical, environmental or host factors.[28] Alterations to the flora may cause abnormal physical conditions or diseases. In the present paper, growth responses to the intestinal microorganisms in *in vitro* were investigated for the extracts from *P. ginseng* and five other oriental medicinal Araliaceae plants.

Among the various human intestinal microorganisms, bifidobacteria are often taken as useful indicators of human health under most environmental conditions. This is based upon the facts that they play important roles in metabolism such as amino-acid production,[26,28] aid defense against infection,[19] are associated with longevity,[18,19] pathogen inhibition[8,9] and immunopotentiation.[3,28] Bifidobacteria

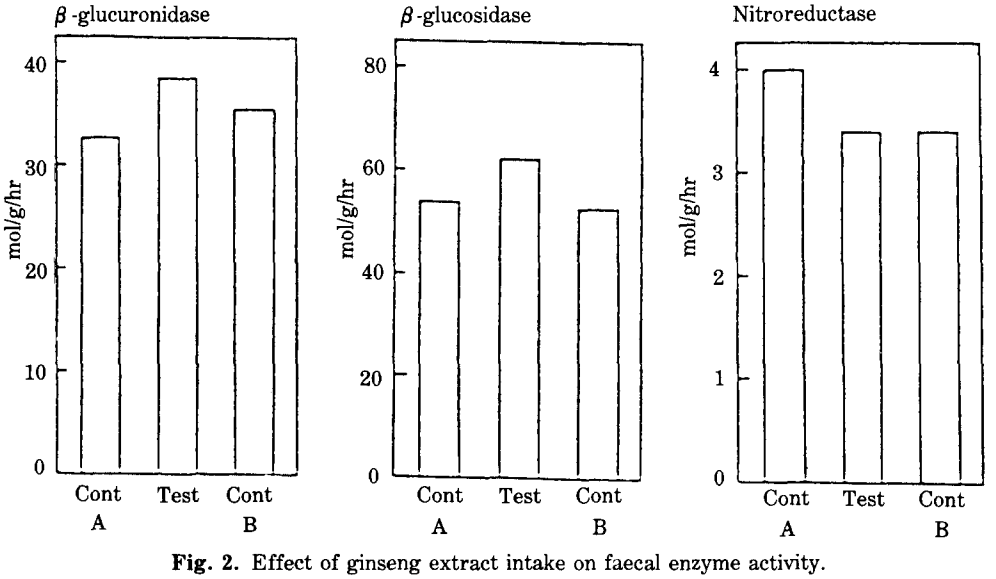

Fig. 2. Effect of ginseng extract intake on faecal enzyme activity.

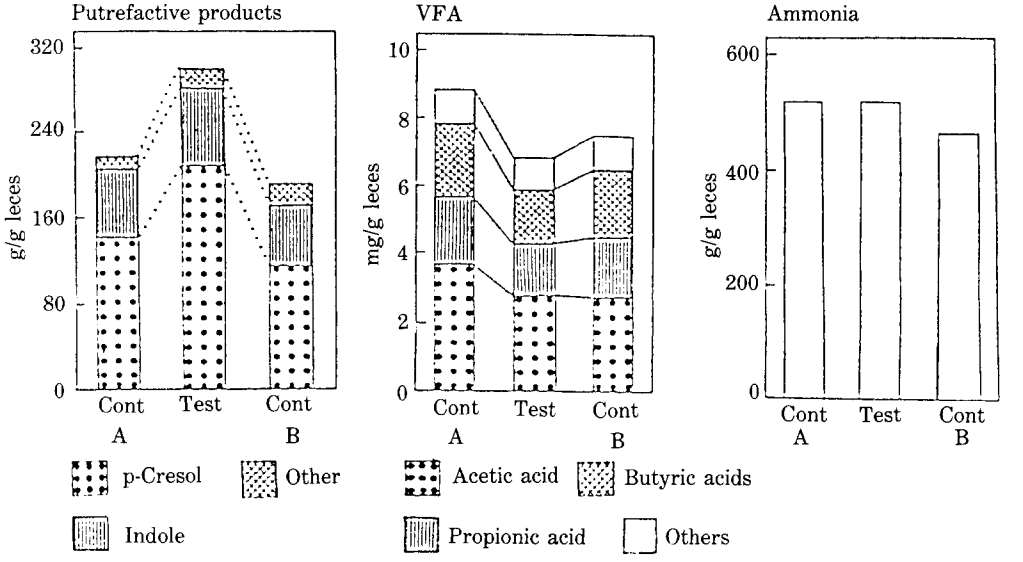

Fig. 3. Effect of ginseng extract intake on faecal metabolites.

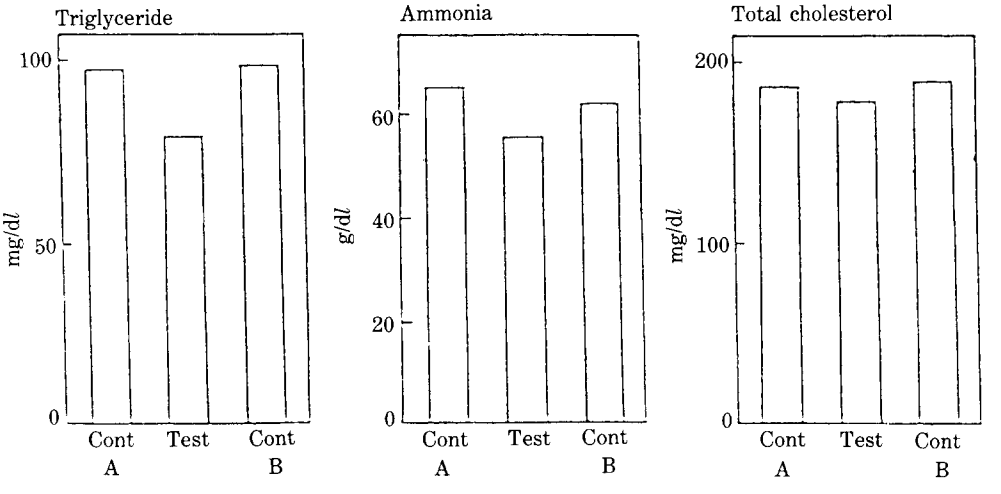

Fig. 4. Effect of ginseng extract intake on blood components.

growth-promoting factors, usually called bifidus factors, have therefore been extentively studied since György *et al.*[17] suggested their existence in human milk. Many oligosaccharides in human milk and N-acetylglucosamine derivatives are growth factors for the organism and also called bifidus factors.[16] Lactulosa, oligosaccharides, peptide or peptide-like and vitamine-like substances have been identified as bifidus factors in human milk,[2,4,5,10,16,21,34] carrot,[37-39] and soybean.[23]

In our microbial assay, addition of extracts from six Araliaceae plants to carbon source-free or carbon source-containing media enhanced the growth of *B. breve* and *B. longum*, indicating the presence of bifidus factor(s) in the extracts.

However, the bifidus factors mentioned above were effective on only some strains of bifidobacteria. There are large variations in the bifidus flora in individuals.[18,29,30] It would be desirable to both inhibit the growth of potential pathogens and/or in-

crease the numbers of many kinds of bifidobacteria in the human gut. Therefore, the growth response of various strains of bacteria to *P. ginseng* was investigated.

The present work revealed that PWE showed strong or moderate growth-promoting activities for many strains of *B. adolescentis, B. longum, B. breve* and *B. infantis,* although it showed no effect on the growth of all strains of *B. bifidum* tested. Also, it was ineffective for the growth of other bacteria including clostridia and *E. coli.* However, *in vivo* experiments using seven healthy adults, the number of *Bifidobacterium* spp. during the period of ginseng extract intake was significantly unaffected from the preceding and subsequent control peroids. This inconsistency between *in vitro* and *in vivo* results may be attributable to the dosage (600 mg ginseng extract/day/2 weeks) used in this study. The much higher intake of ginseng extract might increase the number of *Bifidobacterium* spp.

Differences in intestinal bacteria have been proved between patients and healthy subjects,[11,13] and between younger subjects and elderly subjects.[18,29] For example, the microflora of cancer patients, patients with Alzheimer disease or elderly subjects is known to be mainly composed of clostridia and eubacteria, with a few lactic acid bacteria. Clostridia not only causes conditions with a broad spectrum of clinical severity ranging from mild illness to sudden death, but also contributes significantly to the genesis of various disease states such as toxicity, mutagenesis, carcinogenesis, and aging.[1,12,19,25,28] It acts by biotransforming a variety of ingested or endogenously formed compounds to harmful agents like *nitroso* compounds or aromatic steroids within the gastrointestine.

In the present paper, ginseng extract, selectively inhibit both the growth certain strains of clostridia and frequency of occurrence of subjects having *C. perfringens.* Based upon our data and these earlier findings, this naturally occurring inhibitor could prove useful as new preventive agents against the pathogenic or nonpathogenic disease caused by clostridia such as cancer, Alzheimer disease, and aging.

If the ginseng extract was utilised by some of the intestinal bacteria in human gastrointestine, volatile fatty acids and lactic acid, putrefactive products, and ammonia in faeces would have been lowered.[7,36] However, these biochemical parameters did not changed, suggesting that ginseng extract might not be utilised by some of the intestinal bacteria. In *in vitro* experiments, lactic acid bacteria selectively had ability to utilise it for growth. Therefore, ginseng had selective growth promoting activity for certain intestinal bacteria.

Pharmacological or clinical efficacy of *P. ginseng* to a variety of diseases has been reported.[22,24] It is also well known that *P. ginseng* is an excellent adaptogen. Brekhman and Dardymov[6] established the concept of the tonic effect of *P. ginseng,* suggesting that it normalises disturbed physiological functions rather than treats a specific disease. The pharmacological actions of panax may be explained at least partly by the effect on intestinal microflora. Based upon our data, the intake of *P. ginseng* would be expected to alter the growth and composition of the intestinal microbial community and to modulate the generation of potentially harmful agents within the intestinal tract. This pharmacological effect may also affect toxicity, carcinogenesis, aging and other processes in which intestinal microorganisms participate.

Further work to identify the biologically active substance of *P. ginseng* is in progress.

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