J. Microbiol. Biotechnol. (2002), **12**(4), 657–663

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Effects of *Bacillus polyfermenticus* SCD Administration on Fecal Microflora and Putrefactive Metabolites in Healthy Adults

PARK, KYU-YONG, HWANG-YEONG JUNG, KANG-LYUNG WOO, KYOUNG-DONG JUN[1], JAE-SEON KANG[1], AND HYUN-DONG PAIK*

Division of Life Sciences, Kyungnam University, Masan 631-701, Korea
[1]R&D Center, BINEX Inc., Ltd., Pusan 604-040, Korea

Received: April 9, 2002
Accepted: July 25, 2002

Abstract Probiotics have been suggested to improve gastrointestinal health in humans. To investigate the effects of *Bacillus polyfermenticus* SCD administration on fecal microflora and putrefactive metabolites in humans, *Bacillus polyfermenticus* SCD (4.00×10^5 CFU/mg) was administrated to ten healthy subjects (5 men and 5 women, average age 24 years) three times a day for 2 weeks. Fecal samples were collected before (1st and 2nd weeks, control), during (3rd and 4th weeks), and 2 weeks after the administration. The following microbial groups were evaluated in the feces: aerobic and anaerobic bacteria, *Bacillus polyfermenticus* SCD, *Lactobacillus*, *Bifidobacterium*, total lactic acid bacteria, *Salmonella*, *Clostridium*, *Clostridium perfringens*, *Eubacterium*, *Staphylococcus*, Coliform bacteria, *Pseudomonas*, and Yeast. Fecal concentrations of total aerobic bacteria ($p<0.05$, $p<0.01$, 3rd and 4th weeks), total lactic acid bacteria ($p<0.01$, 3rd, 4th and 5th weeks), and *Bifidobacteria* ($p<0.05$, 4th and 5th weeks) were significantly increased in all subjects, compared to the control, from the 3rd week after the administration of the products. *Clostridium* ($p<0.01$, 4th week), *Clostridium perfringens* ($p<0.05$, $p<0.01$, 3rd and 4th weeks), and coliform ($p<0.01$, 5th week) were significantly reduced from the 3rd week of administration. No significant changes in the fecal concentrations of *Pseudomonas*, *Lactobacillus*, *Eubacterium*, *Staphylococcus*, yeast, and total anaerobes were observed. Six weeks after the administration, the concentration of all microorganisms returned to the basal level. *Bacillus polyfermenticus* SCD was significantly maintained from the 3rd week to 6th week of the study. Despite the absence of a statistical significance, the putrefactive metabolites (ammonia, indole, skatole, and p-cresol) and the pH value tended to be lower during and after the test periods than the base line. These results show that this probiotic preparation is able to colonize the intestine, and suggest that it may be useful as a beneficial probiotic in humans.

*Corresponding author
Phone: 82-55-249-2689; Fax: 82-55-243-8133;
E-mail: hdpaik@kyungnam.ac.kr

Key words: Probiotics, *Bacillus polyfermenticus*, administration, fecal microflora, putrefactive metabolite

The use of probiotic strains for prevention and treatment of intestinal disorders has been suggested for many years [15, 25]. To qualify as a probiotic, bacterial strains must satisfy a number of criteria, including the ability to survive in the gastrointestinal tract [14]. Many studies have provided evidence that some bacterial cultures reduce the risk of some types of cancer and inhibit the growths of certain tumors and tumor cells [17]. Commonly used probiotics are beneficial bacteria to humans, such as *Bifidobacterium* and *Lactobacillus*. These genera are considered to be common anaerobic bacteria of the human fecal flora and to be important components of the normal intestinal flora that represent a complex ecological system. Recently, many articles have been published concerning the beneficial effects of these bacteria on its host. Therefore, it is believed that a large number of lactic acid bacteria (LAB) in the human intestine are important for the health and welfare of the host [1, 2, 3]. On the other hand, the *in vivo* microbial effects of *Bacillus* spp. administration in the intestinal tract have received relatively little attention.

The genus *Bacillus* includes a variety of industrially important species, which are commonly used as hosts in the bioindustry. In particular, *B. polyfermenticus* SCD, which is known as the "Bisroot" strains, have been appropriately used for the treatment of long-term intestinal disorders, since the live strains in the form of active endospores can successfully reach the target intestine [8, 12, 16].

Putrefactive metabolites, such as ammonia, indole, skatole, and p-cresol from undesirable microorganisms, are of great interest in the study of the feces, because they may represent the level of intestinal putrefaction. These

metabolites are formed from urea and amino acid by the action of urease and tryptophanase, respectively [20].

The goals of this study were to evaluate the effects of *B. polyfermenticus* SCD administration on the ecology of human intestinal bacteria and to examine changes in putrefactive metabolites after the intake of *B. polyfermenticus* SCD.

MATERIALS AND METHODS

Subjects

Ten healthy human subjects maintained their usual lifestyles and dietary intakes throughout the study period. The subjects were between 22 and 26 years of age, and there were five males and five females. Their diet was unrestricted, except that dairy products were not consumed. None of the subjects received antimicrobial agents during the study period.

Administration of *B. polyfermenticus* SCD

Ten healthy subjects were given two tablets of *B. polyfermenticus* SCD preparation after one of three usual meals per day for two weeks. Viable *B. polyfermenticus* SCD in the tablet preparation was 1.667×10^7 CFU.

Fecal Sampling Procedures

Fecal samples were collected before (1st and 2nd weeks), during (3rd and 4th weeks), and 2 weeks after administration of the products. Two fecal samples (two per subject), obtained one to six weeks apart, were examined. The samples were collected in sterile plastic containers and immediately taken to the laboratory, where they were separately introduced into an anaerobic and aerobic chamber for the processing of anaerobes and aerobes. On average, processing began within 10 min of collecting the samples.

Microbiological Procedures

One gram was removed from the middle of the fecal sample and was used to make a fecal homogenate in 9 ml of pre-reduced sterilized anaerobic buffer solution (0.85% NaCl, 0.1% L-cysteine, and 0.1% sodium thioglycolate) with substituted gas (CO_2 90%) [9]. A dilution series (10^{-1} to 10^{-7}) was then made in the same medium, and 100 µl aliquots at each dilution were used to inoculate media by the spread plate method. The following media were used for the isolated intestinal microorganisms [9, 13, 20]; dilutions were plated onto EG agar and BL agar for total anaerobes, TS agar for total aerobes, tryptic soy agar for *B. polyfermenticus* SCD, m-LBS agar for *Lactobacillus*, BCP agar for total LAB, SS agar for *Salmonella*, BS agar for *Bifidobacterium*, PO agar for *Clostridium*, NN agar for *Clostridium perfringens*, ES agar for *Eubacterium*, PEES agar for *Staphylococcus*, deoxycholate agar for Coliform bacteria, *Pseudomonas* agar for *Pseudomonas*, and yeast was enumerated by using potato dextrose agar. In addition, dilutions heated at 80°C for 10 min were spread on tryptic soy agar to determine the *B. polyfermenticus* SCD spore count. All plates were incubated for 48 h at 37°C in an anaerobic jar and an aerobic incubator (Coliform bacteria, *Pseudomonas*, 24 h). After incubations, the different colony types were counted and isolated as pure cultures. All isolates were analyzed according to the Gram-stain, catalase

Table 1. Media used for counting human fecal microflora.

Media	Organisms usually enumerated	Incubation method	Incubation temperature (°C)	Incubation period (h)
Nonselective Media				
EG, BL	Strictly anaerobic bacteria	Steel Wool Method	37	48
TS[a]	Aerobic bacteria	Aerobic condition	37	48
TSA[b]	*Bacillus polyfermenticus*	Aerobic condition	37	48
Selective Media				
m-LBS[c]	*Lactobacillus*	Gaspak system	37	48
BCP	Lactic acid bacteria	Gaspak system	37	48
SS	*Salmonella*	Gaspak system	37	48
BS	*Bifidobacterium*	Steel Wool Method	37	48
PO	*Clostridium* (*lecithinase*, -)	Steel Wool Method	37	48
NN	*Clostridium perfringens*	Steel Wool Method	37	48
ES	*Eubacterium*	Steel Wool Method	37	48
PDA[d]	Yeast	Aerobic condition	37	48
PEES	*Staphylococcus*	Aerobic condition	37	48
Desoxycholate agar	Coliform	Aerobic condition	37	24
Pseudomonas agar	*Pseudomonas*	Aerobic condition	37	24

[a]TS: Trypticase soy blood agar.
[b]TSA: Tryptic soy agar.
[c]m-LBS: LBS 84 g, Lab-Lemco powder 8 g, sodium acetate trihydrate 15 g, and acetic acid 3.7 ml in 1,000 ml DDW.
[d]PDA: Potato dextrose agar. EG, BL, TS, m-LBS, BS, PO, NN, ES, and PEES were made according to the method of Benno and Mitsuoka [2].

reaction, oxidase reaction, and morphology by using the API kit. Thereafter, the isolates of the microorganism in the feces were identified to the genus level by using the method of Mitsuoka [4, 5, 18, 19]. Microbiological procedures are summarized in Table 1.

Determination of Fecal pH

Fecal pH was determined at time intervals mentioned above (model HandyLabl; Schott Glaswerk, Mainz, Germany) by inserting pH meter electrode (model BL21; Schott Glaswerk, Mainz, Germany) twice directly into the fresh undiluted fecal sample [10].

Analysis of Putrefactive Metabolites in Feces

Putrefactive metabolites (indole, skatole, and ρ-cresol) in the feces were analyzed using a HP 5890 Series H Plus gas chromatograph (Hewlett Packard Co., U.S.A.). Analytical grade (>99% purity) of indole, skatole, ρ-cresol, and 4-isopropylphenol (internal standard, IS) were obtained from Aldrich (Milwaukee, U.S.A.). The column (DB-17 capillary column) was purchased from J&W Scientific (Folsom, U.S.A.). Fresh fecal samples were frozen immediately after collection and stored at -20°C until processing [22]. Fecal samples were prepared by suspending 2 g of sample in 8 ml of distilled water (pH 9.0), to which was added an internal standard at the level of 50 ppm in 10 ml. After a while, 1 ml of the homogeneous suspension was transferred to a conical polypylene micro sample tube (Eppendorf, 1 ml) and then centrifuged for 10 min at 12,000 rpm and 4°C in a Micro 17R centrifuge (Hanil Science Industrial Co., Ltd., Korea). The supernatant was placed in an Eppendorf tube and 1 μl was injected into the gas chromatograph. For gas chromatographic analysis, 50 ppm of standard materials were processed in the same manner as described above.

After gas chromatography, the mass spectra of the fecal putrefactive metabolites were obtained using an HP 5917 mass-selective detector operated in the electron impact (EI) ionization mode. The ionization energy used was 70 eV, and a DB-17 capillary column (30 m×0.32 mm I.D., 0.25 μm film thickness) was used. Flow-rate of carrier gas (helium) was 0.9 ml/min, and other conditions were as detailed for gas chromatography. The conditions of the GC are summarized in Table 2. The following equation was used to calculate the concentration of the putrefactive metabolites.

$$A\ ppm = \frac{50\ ppm \times \text{area of B in sample solution} \times \text{area of IS in standard solution} \times 10}{\text{area of IS in sample solution} \times \text{area of B in standard solution} \times 2}$$

A: Real concentration of putrefactive metabolites
B: Putrefactive metabolites (indole, skatole, and ρ-cresol)

The quantity of ammonia in the feces was determined using an Ammonium-Test (Darmstadt, Germany).

Statistical Analysis

The average number of microorganism was calculated by using the students t-test to determine significant differences between the treatment and control groups [6, 7, 23]. pH value and putrefactive metabolites were also determined by the same method. A significant difference was defined as $p<0.05$ and $p<0.01$. In the case of microorganisms, data were expressed as mean log 10 number per gram of feces±s.d. In the case of putrefactive metabolites, data are expressed as ppm±s.d.

RESULTS AND DISCUSSION

Effect of *Bacillus polyfermenticus* SCD on Human Fecal Microflora

Fecal samples from 10 subjects were submitted to the laboratory during the study period. As shown in Table 3, fecal concentrations of total LAB, *Bifidobacterium*, and total aerobic bacteria increased significantly in the subjects after administration and returned to control levels at the last week. In particular, total LAB was significantly higher than the control week. High numbers of LAB were

Table 2. Conditions of gas chromatography for analysis on the DB-17 capillary column.

GC	HP 5890 Series H Plus gas chromatograph (Hewlett Packard Co., U.S.A.)
Column	DB-17 capillary column (J&W Scientific), 30 m×0.32 mm, thickness 0.25 μm (91 Blue Ravine Road, Folsom, CA, U.S.A.)
Carrier gas	H_2
Flow rate	1.2 ml/min (Constant EPC flow)
Splitless	Off: after injection at inlet valve for 0.1/min; On: remaining time
Make up gas	30 ml/min
Detector	FI
Initial temp.	80°C
Final temp.	175°C
Temp. programming	5°C
Injection temp.	260°C
Detector temp.	260°C

Table 3. Effect of the administration of *Bacillus polyfermenticus* SCD on the fecal microflora of 10 subjects.

Microorganisms	No. of microorganisms/g feces[a]					
	1st week	2nd week	3rd week[b]	4th week[b]	5th week	6th week
Total anaerobes	9.69±0.40	9.81±0.43	9.49±0.36	9.65±0.49	9.66±0.38	9.65±0.34
Total aerobes	8.32±0.54	8.38±0.64	8.83±0.39*	9.17±0.53**	8.41±0.32	8.54±0.25
Lactobacillus	6.60±0.77	6.65±0.69	6.75±0.76	6.35±1.01	6.80±0.65	6.92±0.55
Lactic acid bacteria	8.42±0.59	8.67±0.57	9.25±0.45**	9.48±0.21**	9.28±0.45**	8.51±0.56
Salmonella	4 (10)[c]	3.6 (10)	2.0 (10)[c]	4.48 (10)	3.18 (10)[c]	5.45 (10)
Bifidobacterium	8.91±0.50	8.98±0.34	9.21±0.18	9.38±0.31*	9.33±0.54*	9.09±0.30
Clostridium (lecithinase, -)	7.17±0.64	7.76±0.74	6.82±1.03	6.63±0.84**	7.16±0.85	6.92±0.79
Clostridium perfringens	5.08±0.98	4.65±0.78	3.89±1.04*	3.83±0.35**	4.13±0.86	4.69±0.57
Eubacterium	7.83±1.08	7.83±0.75	7.68±0.74	7.82±0.88	7.68±0.44	7.68±0.23
Yeast	3.07±0.74	3.44±0.62	3.19±0.79	3.34±0.78	2.83±0.60	3.56±1.39
Staphylococcus	3.21±0.56	3.13±0.56	3.06±0.64	2.77±0.54	2.79±0.53	3.46±0.64
Coliform	7.12±0.55	6.86±0.75	6.76±1.01	5.74±0.73**	6.54±1.11	6.92±0.78
Pseudomonas	3.89±0.47	4.11±0.87	3.52±0.65	3.98±0.44	3.66±1.06	4.07±0.79
Bacillus polyfermenticus	N.T	N.T	5.27±0.59	5.48±0.71	4.18±0.78	3.75±0.67

[a]Mean (log CFU/g feces)±S.D.
[b]*Bacillus polyfermenticus* SCD were administrated three times a day for 2 weeks.
[c]Frequency of occurrence.
[d]Not tested. *$P<0.05$. **$P<0.01$.

maintained until the 5th week of administration. Many studies have found that high numbers of *Bifidobacterium* or LAB in human intestine are important for the host's health and welfare [18], and *B. polyfermenticus* SCD strain was found to be effective in improving beneficial microorganisms in human intestine.

Clostridium spp. ($p<0.01$, 4th week), *Clostridium perfringens* ($p<0.05$, $p<0.01$, 3rd and 4th weeks), and Coliform ($p<0.05$, 4th week) were significantly reduced in the subjects, compared to their control week, from the 3rd week of administration and returned to control level during the last week. It has been reported that fecal *Clostridium*, i.e., *C. butryricum*, *C. innocuum*, *C. indolis*, *C. paraputrificum*, *C. tertium*, and *C. sartagoforme*, constitute a high proportion of fecal microflora in patients with colon cancer. It was also found that *Clostridium* spp. are capable of exhibiting nuclear dehydrogenation, an important reaction in the formation of carcinogens from bile acid [2]. Moreover, in this study, a decreased number of *Clostridium* spp. and *Clostridium perfringens* after *B. polyfermenticus* SCD administration might indicate prevention of intestinal putrefaction and production of carcinogens by these microorganisms. Coliform bacteria are also responsible for undesirable reactions in the intestinal tract [4, 20]. We also found decreasing proportions of coliform bacteria at the 4th week.

No significant changes in the fecal concentrations of *Lactobacillus*, *Eubacterium*, *Staphylococcus*, *Pseudomonas*, *Salmonella*, yeast, and total anaerobes were found. However,

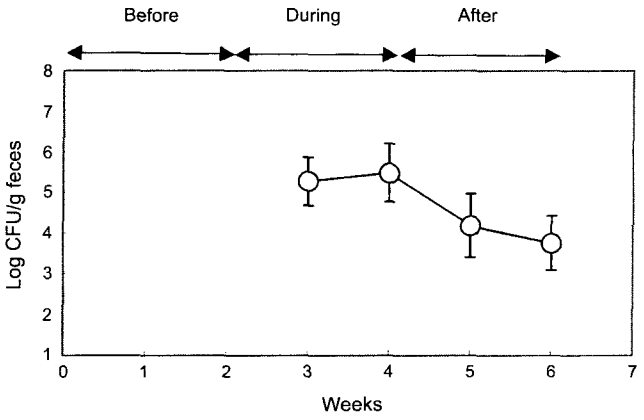

Fig. 1. The number of *Bacillus polyfermenticus* SCD in the feces of 10 subjects.
○, *Bacillus polyfermenticus* SCD.

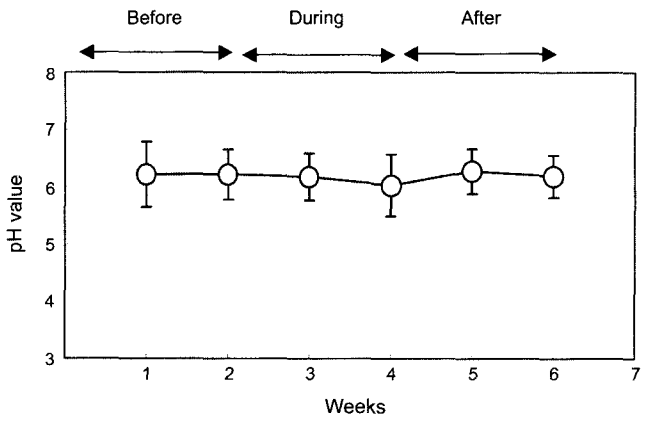

Fig. 2. Variation of fecal pH observed during the study.
○, pH value.

Table 4. Effect of the administration of *Bacillus polyfermenticus* SCD on the fecal putrefactive metabolites of 10 subjects.

Putrefactive metabolites	Concentration[a]		
	Before	During[b]	After
Ammonia[c]	550.00±141.42 (100)[e]	530.00±169.71 (100)	505.00±134.35 (100)
Indole[d]	47.03±28.37 (100)	31.13±21.11 (100)	37.17±29.90 (100)
Skatole[d]	36.61±7.33 (20)	34.59±40.47 (20)	8.90±12.58 (20)
p-Cresol[d]	210.95±133.58 (100)	174.60±139.89 (100)	151.29±103.83 (100)

[a]Mean (ppm feces)±S.D.
[b]*Bacillus polyfermenticus* SCD were administrated three times a day for 2 weeks.
[c]Ammonium-Test (MERCK).
[d]Gas chromatograph.
[e]Frequency of occurrence.

despite the lack of statistical significance, *Staphylococcus* tended to decrease from the 4th week to the 5th week. With an exception of *B. polyfermenticus* SCD, the concentrations of all microorganisms in this study returned to the basal levels by the last week. As shown in Fig. 1, 2 weeks after the administration, the number of *B. polyfermenticus* SCD strain still remained stable.

The present study showed that *B. polyfermenticus* SCD survived passage through the digestive system, indicating their tolerance with low pH of the stomach and the presence of bile in the intestine. Thus, this microorganism is a potential beneficial probiotic strain with the ability to survive in the intestine. As shown in Fig. 2, relatively low pH values in feces with *B. polyfermenticus* SCD were found, and no significant changes occurred during the study. Acidification of the colon prevents the formation of carcinogens or co-carcinogens, such as bacterial degraded-bile acids or cholesterol metabolites [2]. The lower level of

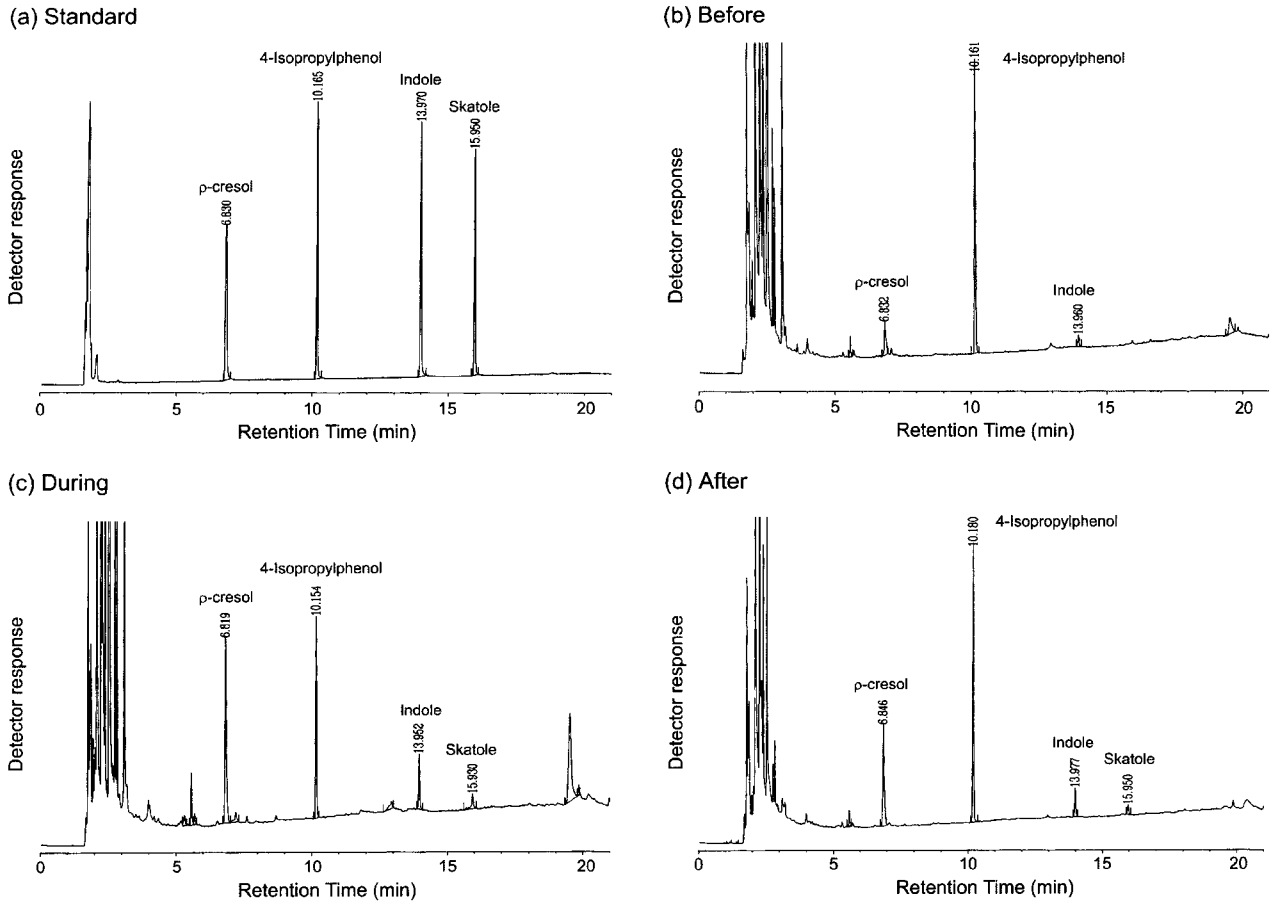

Fig. 3. Gas chromatogram of a standard mixture of putrefactive metabolites and samples of fecal supernatant used in this study.

fecal pH after *B. polyfermenticus* SCD administration might serve as one of the possible protection mechanisms against colonic cancer.

Effect of *B. polyfermenticus* SCD Administration on Fecal Putrefactive Metabolites

Table 4 shows changes in fecal putrefactive metabolites induced by *B. polyfermenticus* SCD administration. There was some relationship between the microbial changes and the levels of ammonia concentrations in the feces. It has already been demonstrated that decrease of *Clostridium* proportion in the total population by *Bifidobacterium* administrations may lower the concentration of fecal ammonia [2]. In this study, a significant decrease of *Clostridium* number and increase of *Bifidobacterium* number were observed after *B. polyfermenticus* SCD administration. Thus, oral supplementation of *B. polyfermenticus* SCD is suggested to be introduced to improve fecal proportions such as fecal ammonia concentration.

In the intestine, indole and skatole are derived from tryptophan by the action of tryptophanase in some putrefactive bacteria. *E. coli*, *Proteus*, *Bacteroides*, and *Clostridium* are representative bacteria with the ability to produce indole. ρ-Cresol is a metabolite derived from tyrosine by some intestinal bacteria. Occasionally, these metabolites in feces have been analyzed, because they may indicate a range of putrefactive microorganisms [10, 20, 24, 25]. Figure 3(a) shows a gas chromatogram of a standard mixture of the putrefactive metabolites (ρ-cresol, indole, skatole, and 4-isopropylphenol; I.S.), and a typical chromatogram of a fecal sample is shown in Fig. 3(b). In this study, ρ-cresol and indole were found to be the main components in the feces, whereas skatole was present less frequently in minor concentrations. Baseline separation was obtained for all metabolites and I.S. within 17 min; peaks were symmetrical with any tailing. However, despite no statistical significance, the ratio of ρ-cresol concentration to indole and skatole in the feces tended to be lower compared to the control week. After analyzing by gas chromatography, the putrefactive metabolites were identified by mass spectrometry. The present study describes a very simple, reproducible, and rapid method for the determination of fecal putrefactive metabolites, by directly injecting fecal supernatants into a gas chromatograph without any pretreatment. This study shows that this beneficial probiotic strain has the potential to enhance intestinal health in humans.

REFERENCES

1. Asahara, T., K. Nomoto, K. Shimizu, M. Watanuki, and R. Tanaka. 2001. Increased resistance of mice to *Salmonella enterica* serovar *typhimurium* infection by symbiotic administration of bifidobacteria and transgalactosylated oligosaccharides. *J. Appl. Microbiol.* **91:** 985–996.
2. Benno, Y. and T. Mitsuoka. 1992. Impact of *Bifidobacterium longum* on human fecal microflora. *Microbiol. Immunol.* **36:** 683–694.
3. Byun, J. W., G. T. Kim, H. S. Bae, Y. J. Baek, and W. K. Lee. 2000. *In vitro* selection of lactic acid bacteria for probiotic use in pigs. *Kor. J. Vet. Res.* **40:** 701–706.
4. Han, B. J., S. K. Woo, and H. K. Shin. 1995. Effects of the water extract of Akebia on the growth of *Clostridium perfringens* and some intestinal microorganism. *Kor. J. Appl. Microbiol. Biotechnol* **23:** 633–640.
5. Jin, H. S. 1997. Fecal microflora of college students in Jeon-Buk area. *Kor. J. Environ. Biol.* **15:** 39–43.
6. Jin, H. S., K. J. Lee, and S. J. Moon. 1999. Relations between the microfloral composition and the environmental factors affecting Korean infants during lactation. *Kor. J. Nutr.* **32:** 64–74.
7. Jun. H. S., Y. K. Choi, Y. S. Won, B. H. Hun, and J. W. Kim. 1999. Effects of lactic acid bacteria on infection of *Salmonella typhimurium* in mouse. *Kor. J. Dairy Sci.* **21:** 171–182.
8. Jun, K.-D., K.-H. Lee, W.-S. Kim, and H.-D. Paik. 2000. Microbiological identification of medical probiotic Bispan strain. *Kor. J. Appl. Microbiol. Biotechnol.* **28:** 124–127.
9. Kang, K. H., K. M. Kim, and S. G. Choi. 1996. Effects of the fructooligosaccharide intake on human fecal microflora and fecal properties. *Kor. J. Food Sci. Technol.* **4:** 609–615.
10. Kim, G. T., Y. I. Hwang, S. I. Lim, and D. S. Lee. 2000. Carbon dioxide production and quality changes in Korean fermented soybean paste and hot pepper-soybean paste. *J. Kor. Soc. Food Sci. Nutr.* **29:** 807–813.
11. Kimoto, H., J. Kurisaki, N. M. Tsuji, S. Ohmomo, and T. Okamoto. 1999. Lactococci as probiotic strains: Adhesion to human enterocyte-like Caco-2 cells and tolerance to low pH and bile. *Lett. Appl. Microbiol.* **29:** 313–316.
12. Lee, K.-H., K.-D. Jun, W.-S. Kim, and H.-D. Paik. 2000. Partial characterization of polyfermenticin SCD, a newly identified bacteriocin of *Bacillus polyfermenticus*. *Lett. Appl. Microbiol.* **32:** 146–151.
13. Lee, W. K., S. M. Lee, H. S. Bae, and Y. J. Baek. 1999. Effect of *Bifidobacterium longum* HY8001 administration on human fecal bacterial enzymes and microflora. *Kor. J. Microbiol. Biotechnol.* **27:** 267–272.
14. Matsuzaki, T. and J. Chin. 2000. Modulating immune responses with probiotic bacteria. *Immunol. Cell Biol.* **78:** 67–73.
15. Nardi, R. D., A. R. M. Santos, M. A. R. Carvalho, L. M. Farias, L. C. Benchetrit, and J. R. Nicoli. 1999. Antagonism against anaerobic and facultative bacteria through a diffusible inhibitory compound produced by a *Lactobacillus* sp. isolated from the rat fecal microbiota. *Anaerobe* **5:** 409–411.
16. Paik, H.-D., N.-K. Lee, K.-H. Lee, Y.-l. Hwang, and J.-G. Pan. 2000. Identification and partial characterization of cerein BS 229, a bacteriocin produced by *Bacillus cereus* BS 229. *J. Microbiol. Biotechnol.* **10:** 195–200.
17. Romond, M. B., A. Yazourh, and C. Romond. 1997. Cell-free wheys from bifidobacteria fermented milks exert a

regulatory effect on the intestinal microflora of mice and humans. *Anaerobe* **3:** 137–143.
18. Ryu, B. H., S. H. Cho, S. W. Ha, K. M. Park, and K. H. Kang. 1998. Changes of the intestinal microflora and fecal properties by intake of yoghurt added capsulated or uncapsulated Bifidobacteria. *Kor. J. Microbiol. Biotechnol.* **26:** 221–225.
19. Shin, M. S., H. M. Kim, G. T. Kim, C. S. Huh, H. S. Bae, and Y. J. Baek. 1999. Selection and characteristics of *Lactobacillus acidophilus* isolated from Korean feces. *Kor. J. Food Sci. Technol.* **31:** 495–501.
20. Shin, M. S., Y. J. Kim, H. S. Bae, and Y. J. Baek. 1996. Effects of the lactic acid bacteria administration on fecal microflora and putrefactive metabolites in healthy adults. *Kor. J. Appl. Microbiol. Biotechnol* **24:** 254–260.
21. Sullivan, A., A. C. Palmgren, and C. E. Nord. 2001. Effect of *Lactobacillus paracasei* on intestinal colonisation of lactobacilli, bifidobacteria and *Clostridium difficile* in elderly persons. *Anaerobe* **7:** 67–70.
22. Tangerman, A. and F. M. Nagengast. 1996. A gas chromatographic analysis of fecal short-chain fatty acids, using the direct injection method. *Anal. Biochem.* **236:** 1–8.
23. Venturi, A., P. Gionchetti, F. Rizzello, R. Johansson, E. Zucconi, P. Brigidi, D. Matteuzzi, and M. Campieri. 1999. Impact on the composition of the fecal flora by a new probiotic preparation: Preliminary data on maintenance treatment of patients with ulcerative colitis. *Ailment Pharmacol. Ther.* **13:** 1103–1108.
24. Yoshihara, I. and K. Maruta. 1977. Gas chromatographic microdetermination of indole and skatole in gastrointestinal contents of domestic animals. *Agric. Biol. Chem.* **41:** 2083–2085.
25. Yoshihara, I. 1978. Gas chromatographic detection of volatile phenols and microdetermination of ρ-cresol in gastrointestinal contents of domestic animals. *Agric. Biol. Chem.* **42:** 1607–1609.